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CONTENTS

The Strength of the Earth's Crust. *By* S. WARREN CAREY 303
Physical Adsorption Processes and Their Relation to Order-Disorder Theory. *By* J. M. HONIG 313
Experimental Yellow Fever. *By* W. D. TIGERTT, T. O. BERGE, W. S. GOCHENOUR, C. A. GLEISER, W. C. EVELAND, C. VORDER BRUEGGE, and H. F. SMETANA 323
The Interdependence Between Instrumental and Chemical Analytical Approaches. *By* SIDNEY SIGGIA 334
Slow Neutron Interactions with Matter. *By* T. J. KRIEGER 339
An Experimental Analysis of the Role of Context in Verbal Behavior. *By* CHARLES N. COFER 341
Tumors as Unique Biological Tools in Basic Biochemical Studies. *By* VERNON RILEY 348
Mycoparasitism of *Gonatobotryum Fuscum* and *Piptocephalis Xenophila*. *By* ALEX L. SHIGO 365
Conferences Held 373
New Members 380

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SECTION OF GEOLOGICAL SCIENCES

THE STRENGTH OF THE EARTH'S CRUST*

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During the last three years geophysicists in three independent fields have concluded, as a corollary to their primary investigations, that the strength of the earth's crust is greater than had been commonly thought. Innes (1957), following Garland (1950), considered that large negative gravity anomalies in central Quebec were due to orogenic roots of Precambrian age and that the large implied isostatic loads had been borne by the crust through geological eras. More recently O'Keefe *et al.* (1959) reported that the orbits of the artificial satellites indicated a north-south asymmetry of the geoid and concluded that this indicated a very much greater strength for the crust over wide arcs than had been accepted by Heiskanen and Vening Meinesz (1958) and geophysicists generally. Finally, Gough and van Niekerk (1959) studied the thermoremanent magnetism of the Bushveld lopolith and observed that the paleomagnetic poles had a significantly smaller scatter when corrected for stratigraphic dip. They interpret this to mean that the crust supported the load due to the intrusion for at least 10^5 years, and concluded that "the South African crust 2×10^9 years ago appears to have had very considerable strength."

Taken together, these independent results tend to reinforce each other and to lead to a general conviction that the crust is strong and load-resistant over very long periods. Already these results are being generally quoted. For example, Garland has reiterated his and Innes's conclusion in the authoritative *Encyclopedia of Physics* (1956), and Hawkes in his anniversary address to the Geological Society (1959) in which he reviewed a half century of progress in geological thought, particularly in respect to the earth's interior, quoted Innes's conclusion verbatim. For these reasons it is necessary to show that none of these conclusions necessarily follows from the data presented.

Gravity Anomalies of the Canadian Shield

The large Bouguer anomalies that have been mapped during the last decade by geophysicists of the Dominion Observatory, Ottawa, Ont., Canada, in the Quebec Province of the Canadian shield are shown on FIGURE 1. Innes (1957) argues that, since the large anomalies occur where there is evidence of an ancient orogenic belt, these anomalies

*This paper, illustrated with slides, was presented at a meeting of the Section on February 1, 1960.

are caused by the residual roots of such an orogenic belt, whose surface mountains have long since been eroded away, and that the strength of crust has been great enough to prevent the mass deficiency represented by the root from forcing up the crust into isostatic equilibrium. In this Innes has followed Garland (1950, pp. 37-45) who based his ideas on the tectogene concept that has since been abandoned as a result of the crustal seismic investigations in the Caribbean region where the tectogene concept was conceived. Neither Garland (1950) nor Innes (1957) discusses the possibility that the anomaly might be connected with the Quaternary glaciation.

I have plotted on FIGURE 1 the direction of flow of the Labrador ice sheet as indicated by the drumlins, striated pavements, and eskers shown on the 1958 Glacial Map of Canada. The center of dispersion of the Labrador ice sheet was near the northern edge of FIGURE 1. Beyond this there is an ice divide and thereafter the ice flow is to the northeast, north, and northwest. There is nothing to be gained at present from extending FIGURE 1 to include these data because the published gravity results do not extend beyond the limits of the figure.

There is the clearest evidence that substantial uplift has occurred and is still currently in progress in this region as indicated by local seismic-

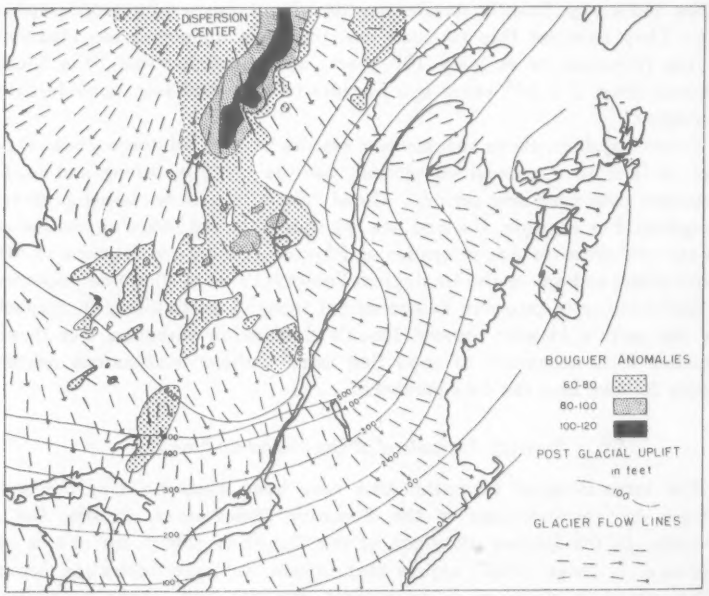

FIGURE 1. Map of southeastern Canada showing Bouguer negative gravity anomalies, flow lines of the Labrador ice sheet, and contours of minimum values of postglacial uplift.

ity. FIGURE 1 shows approximate contour lines of equal postglacial uplift. These indicate minimum uplift, since they are based on present altitudes of marine sediments and strands; the actual total uplift could be greater. The uplift of the central region is substantially greater than the greatest contour shown, but no marine sediments are available to delineate the higher uplift contours.

Two facts stand out in FIGURE 1. The large general negative anomaly is centrally and axially located over the site of the former Labrador ice sheet, and the lines of postglacial uplift frame the present anomaly. The anomaly is located exactly where it ought to be if it is due to the incomplete isostatic recovery of the crust following the melting of the Labrador ice sheet. Guided by recent seismic soundings in Greenland and Antarctica, we could reasonably assume an ice thickness of 3 km. for the central area of the Labrador ice sheet. If we assume that isostatic recovery has been proceeding since the melting of the ice sheet about 10,000 years ago and is already about one third complete, there would now be a residual negative isostatic anomaly of the order of 60 to 80 milligals, with a rising gradient to zero toward the margins of the former ice cap. This is just what Innes's study has found (the anomalies shown on FIGURE 1 are Bouguer anomalies, which on applying isostatic correction are reduced to a maximum of 80 milligals). This would imply that the central area has already risen some 400 m. and that a further uplift of twice this amount will occur at a diminishing rate before isostatic equilibrium is complete.

Apart from the magnitude and distribution of the anomaly, the gradient is relevant in that it might limit the possible depth of the mass deficiency. Garland has pointed out that some of the gradients are steep. However, it must be remembered (as Garland himself has stated) that all masses and mass irregularities in the region contribute to the resulting anomaly. The anomaly due to the partially recovered glacial depression should be broad with gentle gradients since the deficiency is large and its location must be at the base of the depressed layers. Superimposed on this are the anomalies due to rock distribution, particularly the large areas of greenstones, schists, and granites, which differ substantially in density. Anomalies due to the distribution of these rocks may steepen substantially the glacial gradient, and give it a false appearance of being due to a shallow cause. The regional topographic gradient also steepens the gradient of negative Bouguer anomalies since, in the absence of any isostatic anomaly, the Bouguer anomalies reflect the orographic pattern.

Fischer (1959) has studied the relations of the Pleistocene glaciation on a much broader scale. He finds that, although substantial isostatic recovery has already occurred in all areas, there is a general close correlation between recently glaciated areas and depressions of the geoid. The current rate of recovery is of the order of 3 m. per century

in the Hudson Bay area, where the present residual depression of the geoid is of the order of 30 m. Innes's interpretation of the Quebec anomaly is not consistent with Fischer's conclusions.

Far from indicating that the crust has borne large loads through several geological eras, Innes's data are consistent with a substantial degree of isostatic recovery in 10,000 years in one of the cold stable regions of the earth's crust.

Remanent Magnetism of the Bushveld Gabbro

The recent investigation by Gough and van Niekerk of the thermoremanent magnetism of the Bushveld lopolith follows an earlier report by Gough (1956) on the paleomagnetism of the Pilansberg dykes that determined the position of the north magnetic pole at the time of the Pilansberg intrusion to have been in eastern Abyssinia at 7½°N 42½°E. The 6 sampling sites from the Bushveld lopolith showed a wide scatter throughout the African continent (FIGURE 2*a*), but when the pole positions determined from each site are displaced as they would be if the stratigraphic dip at each site were returned to horizontal, the scatter is substantially reduced (FIGURE 2*b*). From this Gough and van Niekerk conclude that the sagging of the lopolith to basin shape took place after the intrusion had cooled through its Curie point. Using Jaeger's formula for the rate of cooling of thick igneous sheets, they conclude further that the crust must have supported the additional load of the intrusion for at least 10^5 years, thereby implying considerable strength for the Precambrian crust.

Accepting for the moment the general assumptions made by Gough and van Niekerk, the case is not as clear as they suggest. Although the pole positions based on the thermoremanent magnetism of the Bushveld gabbro have a smaller scatter when completely corrected for structural dip than have the poles uncorrected for dip, an equally good reduction of scatter is obtained by correcting, for example, three quarters of the dip (FIGURE 2*c*). The smallest circle that will include all the poles within the limits of their probability fields is the same for three-quarters correction as it is for full correction. However, if the Pilansberg pole represents the same general igneous epoch (as seems probable), then the three-quarters or even the half correction gives a more probable distribution than the full correction, which appears to be substantially overcorrected. This could mean that the sagging commenced well before the temperature passed through the Curie point. Since the heat loss is most rapid in the earlier stages of cooling, this would mean that local sagging may have commenced quite early. Furthermore, Gough and van Niekerk's scatter figures take into account only the statistical probability fields related to the scatter of the magnetic vectors, whereas the text indicates that there is significant uncertainty in the readings of

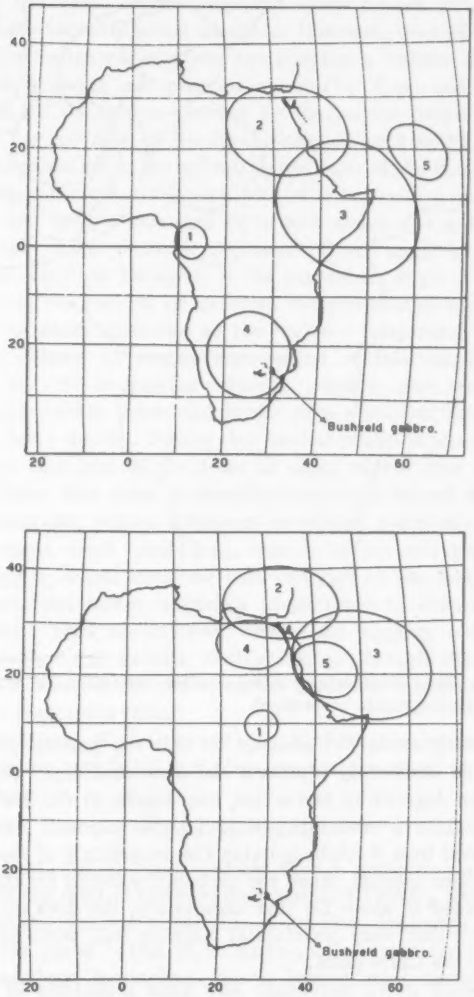

FIGURE 2*a*. Paleomagnetic poles corresponding respectively to 5 sampling sites in the Bushveld Gabbro. Reproduced from Gough and van Niekerk by permission of Philosophical Magazine, London, England.

FIGURE 2*b*. Same Paleomagnetic poles after correction for structural dip. Reproduced from Gough and van Niekerk by permission of Philosophical Magazine, London, England.

FIGURE 2c. Same Paleomagnetic poles after correction for three fourths of structural dip (stereographic projection).

dip, even setting aside the assumption that the parting planes of the igneous rock do accurately represent the structural dip. Probable errors of up to seven degrees in strike and one degree in dip are given. It is unreal to calculate a probability field for the magnetic vector and for the pole deduced from it while ignoring the uncertainty of the dip through which it has been rotated. When the probability fields for the pole positions are widened to allow for this uncertainty, the data do not exclude the possibility that very substantial sagging had occurred before the rock cooled to the Curie point.

However, even if we set aside any such criticisms of the details of the investigation, the underlying assumptions that the present basin shape of the Bushveld body is attributable entirely or largely to the weight of the gabbro is not by any means commanding. The Transvaal System into which the gabbro is intruded forms a typical sedimentary basin, a type of crustal structure that quite characteristically suffers steady or intermittent basin subsidence over times of the order of 10^7 to

10^8 years. Why should postintrusion basin sagging in such an environment imply that the crust had borne a load? Several plausible mechanisms for such an intrusion can be suggested which do not involve an excess load on the sedimentary prism involved. However, even if we accept the view that the intrusion involves transferring a volume of magma from below a crustal layer to a position above that layer and that the layer must sag to fill the space vacated by the magma below it, we should still expect a great deal of the final observed sagging to have occurred after the consolidation of the gabbro in the case of the Bushveld lopolith, for the Bushveld pluton is a multiple intrusion with a very large body of cognate red granite intruded after and above the gabbro. Even in a strengthless crust, at least as much of the sagging in the gabbro should occur after the intrusion of the granite as might occur following the intrusion of the gabbro. In addition, there should be regional sagging for millions of years afterward as the regional temperature declined. To produce the volume of magma represented in this intrusion (at least 100,000 cu. km.) the underlying subcrust, whence came the magma, must have been appreciably hotter and hence more expanded than normal subcrust at the same depths. During this heated phase isostatic equilibrium would require this part of the crust to stand higher than average crust elsewhere. When this zone eventually cooled to normal it should contract, the mass per square kilometer remaining substantially constant. Hence the upper crust would sag, and it is not unexpected that the maximum sagging would coincide with the site of the largest intrusion. This plutonic heat would require a longer time to dissipate than the intrusion would need for cooling. Hence the sagging should continue after both gabbro and granite bodies cooled through the Curie point. However, this does not indicate that the crust has borne any significant load for any appreciable time.

The observations of Gough and van Niekerk are interesting, but it is reasonable to conclude that they cannot be cited as evidence of crustal strength.

Satellite Orbits

O'Keefe *et al.* (1959) have reported that a periodic variation of the eccentricity of the orbit of the Vanguard satellite $1958\beta_2$, which coincided in period with the rotation of the line of apsides, indicates a third zonal harmonic of the geoid – that is, a north-south asymmetry in the earth's figure; the northern hemisphere has a longer axial semidiameter, but a smaller mid-latitudinal semidiameter than the southern hemishpere: in other words the earth is slightly pear-shaped. Earlier, King-Hele and Merson (1958) had suggested that a north-south asymmetry might explain perturbations observed in the orbit of Sputnik 2. Recently O'Keefe has presented the latitude plots of the second, third, and fourth harmonics in terms of geoid height, deflection of the vertical, and milligals of

departure of the external gravitational field from a spherical isopotential. In interpreting the significance of the anomaly O'Keefe shows that if the asymmetry of the geoid were due to past disturbances now in process of slow viscous recovery toward fluid equilibrium, the life of the disturbances would be of the order of 10^5 years and would imply current sea-level changes at a rate much greater than those observed. He concludes that the "zonal harmonics must be supported either by ordinary mechanical strength in the earth's interior or perhaps by hydrodynamic forces such as those resulting from convection."

There is another alternative, namely, that significant variations exist in the density of the mantle, and that the observed geoid is in isostatic equilibrium within the limits previously suggested by Heiskanen and Vening Meinesz. Substantial variations of seismic velocity have been observed for the uppermost mantle rocks immediately below the Mohorovicic discontinuity. Munk (1958) has reported that, in the absence of such density variations in the mantle, the pole should be moving toward a stable position near Hawaii and should have arrived there in a time of the order of 10^4 years, whereas paleomagnetic observations indicate a much higher order of stability, and that the long-term secular movement of the pole has been in the opposite direction. The order of magnitude and qualitative distribution of mantle density variation required to give the observed stability of the pole in opposition to the inertial effects of the continents is similar to that required to produce the asymmetry of the geoid observed by O'Keefe and his colleagues.

Furthermore, the north-south asymmetry of the geoid revealed by the satellite orbits is to be expected from the asymmetrical expansion of the globe deduced by me on geotectonic grounds (Carey 1958, 1959, and 1959*a*). According to the expanding earth hypothesis, less dense mantle and a geoid surface above the theoretical spheroid might be expected in areas of post-Paleozoic orogenesis and below "new" oceans (Arctic, Atlantic, and Indian Oceans, the Mediterranean Sea, and the Pacific Ocean west of the andesite line), and a geoid depressed below the spheroid and regionally denser mantle might be expected under the older continental blocks and the older oceanic segments (about one half the present Pacific Ocean). Taken collectively, these large areas of one-sign anomaly, when measured against a rotational symmetrical spheroid, would produce a geoid that would be pear-shaped when generalized to the third zonal harmonic. However, I expect that further refinements in the determination of the external gravity field will disclose higher-order harmonics indicating asymmetries with respect to octants. Earlier crude geodetic data had indeed already suggested to Heiskanen and others that an elliptical equator might give the best fit to the figure of the earth. The curve of variation with time of the eccentricity of the orbit of satellite $1958\beta_2$ published by O'Keefe *et al.* (1959), departs from the smooth curve predicted by simple north-south

asymmetry, and seems to show forward-pointing cusps due, perhaps, to additional irregularity in the geoid.

My tectonic prediction is that the geoid eventually will prove to have broad nonsystematic irregularities perhaps as large as 30 m., due to regional variations in the upper mantle. However, these will not imply crustal strength of greater order than that suggested by Heiskanen and Vening Meinesz, since they will be in isostatic equilibrium with respect to vertical loads. It is true that such irregularities would imply small stress differences in that the lowest potential energy would be obtained when these irregularities flow laterally to form a uniform layered shell. As O'Keefe has pointed out (1959*a*), perfect fluid equilibrium demands that the density on a given geopotential surface be constant. However, this degree of static equilibrium is not attained even when the isostatic equilibrium of continents, oceans, and mountain ranges is perfect and would not be attained until the continents flow laterally by spreading to form uniform density layers over the whole earth to give perfect density stratification of mantle, intermediate, and granite layers. However, in departures from vertical isostasy the entire load is borne by every cubic centimeter element in the column and adjustment is comparatively rapid, whereas in broad lateral departures such as are involved in the third harmonic the loads implied by the difference in level of the center of gravity of the masses must be divided by their horizontal distance apart in centimeters to get the stress difference across any one element. Accordingly, the stress gradient driving such flow is several orders of magnitude lower than the stresses leading to vertical adjustment. Even the persistence of these gradients over long periods does not necessarily indicate strength, since the half life for viscous decay would be very long indeed. These loads are confined to the niveaux within which the density differences occur and, unlike vertical loads, there is no resulting stress difference in the deeper layers below, which may remain in perfect fluid equilibrium.

Figure of the Moon

Long ago Jeffreys (1929) concluded that the present figure of the moon could be reconciled by supposing that the moon last adjusted itself to the hydrostatic state when its distance from the earth was about 140,000 km. and its period of revolution 6.3 days, and that this implied that the moon's interior must have the strength to support permanently a stress difference of 2×10^7 dynes/sq. cm. By reasonable analogy he concluded that the earth should have similar permanent strength. However, this whole proposition assumes without argument that the density of the moon is symmetrical with respect to both latitude and longitude. Now that asymmetry of figure is established for the earth and asymmetry of development deduced from tectonic data, similar asymmetry is a reason-

able possibility on the moon. Any such asymmetry in either earth or moon would determine the axis of stability. For the earth the axis of maximum inertia would become the axis of rotation and, for the moon, the protuberance would become pointed toward the earth, both indeed as we observe them to be.

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PHYSICAL ADSORPTION PROCESSES AND THEIR RELATION TO ORDER-DISORDER THEORY*

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Survey of the Method

In a series of remarkable papers, Hijmans and de Boer[1] have provided a very systematic approach (the HB method) for the solution of problems in order-disorder theory. Their methodology has been applied here to the determination of gas adsorption isotherms. Only the main features of this method are introduced in the simplified treatment given below; the formal theory will be detailed elsewhere.

We idealize the surface by regarding it as a regular geometric array of sites, termed a lattice, sufficiently extensive so that edge effects may be neglected. Each site (also termed a point) is associated with the adsorption energy $-\epsilon$ and serves as a center for localized adsorption,[2] this process being limited to a monolayer.

In the presence of lateral interactions among adsorbed molecules, the probability of occupation of a given site depends on the state of occupation of all others. To simplify the complexity of the theory, we follow the HB method and decompose the lattice into aggregates of simple figure arrays (subfigures) consisting of but a few sites. Each subfigure may be characterized by a small number of microvariables and thereby becomes amenable to mathematical analysis. The specification of a set of lattice sites that is occupied under a given set of conditions is known as a configuration or occupation state. Every such state must be reflected in appropriate configurations of the subfigures. Once the latter are known, the energy and entropy of the figure arrays may be determined, and the (Helmholtz) free energy may be minimized so as to specify equilibrium conditions. From the latter the isotherm equation can be constructed. The theory will be illustrated in the next two sections.

The Zero-Order Approximation

In this approximation the lattice is regarded simply as a collection of L points of which $a_0 L$ are unoccupied and $a_1 L$ are occupied; the energy of each empty and filled site will be designated by ϵ_0 and ϵ_1. The internal

*This paper, illustrated with slides, was presented at a meeting of the Section on February 2, 1960. The work described in this article was performed by Lincoln Laboratory, a center for research operated by Massachusetts Institute of Technology with the joint support of the United States Army, Navy, and Air Force.

degrees of freedom of the adsorbed molecules will be ignored to rid the theory of all nonessentials.

The energy of the array of subfigures is

$$E = L\{a_0 \epsilon_0 + a_1 \epsilon_1\} \tag{1}$$

and the entropy is given by

$$S = k \ln W = k \ln \{L!/(a_0 L)!\,(a_1 L)!\} \tag{2}$$

Using Stirling's approximation ($\ln n! \approx n \ln n - n$), EQUATION 2 reduces to

$$S = -k L \{a_0 \ln a_0 + a_1 \ln a_1\} \tag{3}$$

and EQUATIONS 1 and 3 may be combined into the free-energy expression

$$F = E - TS = L\{(1-a_1)\epsilon_0 + a_1 \epsilon_1 + kT[(1-a_1)\ln(1-a_1) + a_1 \ln a_1]\} \tag{4}$$

where account has been taken of the normalization condition

$$a_0 + a_1 = 1 \tag{5}$$

The chemical potential of the adsorbed species is, accordingly,

$$\mu_s = \frac{1}{L}\left(\frac{\partial F}{\partial a_1}\right)_T = -\epsilon_0 + \epsilon_1 + kT \ln [a_1/(1-a_1)] \tag{6}$$

Under equilibrium conditions, EQUATION 6 will be equal to the chemical potential of the gas phase[3]

$$\begin{aligned} \mu_g &= -kT \ln (2\pi m\, kT/h^2)^{3/2} j_g\, kT/p \\ &\equiv -kT \ln (p^*/p) \end{aligned} \tag{7}$$

where all symbols have their conventional significance.[3]

If the energy levels are fixed by setting $\epsilon_0 = 0$, $\epsilon_1 = -\epsilon$ and if we define $a_1 \equiv \theta$, then a combination of EQUATIONS 6 and 7 leads to

$$p = p^* e^{-\epsilon/kT} \frac{\theta}{1-\theta} \equiv p^0 \frac{\theta}{1-\theta} \tag{8}$$

which is the Langmuir isotherm, except that the factor p^0 should have read p^0/Q_s, where Q_s is the partition function for the internal degrees of freedom of the adsorbed molecules.[3-5] The correct result would have been obtained had account been taken of the internal degrees of freedom in setting up EQUATIONS 1 and 2.

The result (EQUATION 8) is very reasonable, for in the approximation regarding the lattice as a collection of points, the adsorbed molecule is effectively isolated from all others; under these conditions, the Langmuir equation must be recovered.

The First-Order Approximation

In an extension of the above, let the lattice be regarded as a superposition of "bonds" O–O and "sites" O. If Z represents the coordination number for a lattice point, $(Z/2)L$, nearest neighbor pairs can be formed; this is also the number of bonds required in the lattice representation. Included in these subfigures is the set of ZL points (2 per bond). To rectify the overcount, an array of $(1 - Z)L$ points is set up, so that the totality of $(Z/2)L$ bonds and $(1 - Z)L$ points correctly represents the lattice to the first approximation.

Next, a listing of possible occupation states and energies for the subfigures must be provided; this is done in TABLE 1. With the aid of

TABLE 1

OCCUPATION AND ENERGY STATES FOR MEMBERS IN THE BOND AND POINT FIGURE ASSEMBLIES

Filled Sites Are Represented by Full Circles

Configuration	*Fraction in configuration*	*Energy*
O	a_0	ϵ_0
●	a_1	ϵ_1
O–O	β_0	ζ_0
O–●	$\{2\beta_1$	$\{\zeta_1$
O–●		
●–●	β_2	ζ_2

TABLE 1, it is possible to write out the total energy of the lattice representations as

$$E = L\,\{(Z/2)[\beta_0\zeta_0 + 2\beta_1\zeta_1 + \beta_2\zeta_2] + (1-Z)\,[a_0\epsilon_0 + a_1\epsilon_1]\} \tag{9}$$

The entropy is given by

$$S = k\ell n\,\Omega = k\ell n\left\{\frac{[(Z/2)\,L]!}{(\beta_0\frac{Z}{2}L)!\,[(\beta_1\frac{Z}{2}L)!]^2\,(\beta_2\frac{Z}{2}L)!} \times \frac{[(1-Z)\,L]!}{[(1-Z)\,L\,a_0)]!\,[(1-Z)\,L\,a_1]!}\right\} \tag{10}$$

Considerable simplification is achieved by taking cognizance of the normalization conditions (EQUATION 5) and

$$\beta_0 + 2\beta_2 + \beta_2 = 1 \tag{11}$$

In addition, there exists a consistency requirement

$$\beta_1 + \beta_2 = a_1 \tag{12}$$

that is due to the fact that an occupied point in the bond assembly is represented by a terminus of the bond configuration ●–○ or ●–●.

In applying Stirling's approximation, all nonlogarithmic terms cancel mutually when EQUATIONS 5 and 11 are introduced. The remaining terms are of the form $(L\,\beta_2\,Z/2)\,\ell n\,(L\,\beta_2\,Z/2)$ that may be rewritten as $(L\,\beta_2\,Z/2)\,[\ell n\,(L\,Z/2) + \ell n\,\beta_2]$. When terms in $\ell n\,(L\,Z/2)$ are collected, further cancellations occur because of EQUATIONS 5, 11, and 12.

This leaves

$$S = -kL\,\{(Z/2)\,(\beta_0\,\ell n\,\beta_0 + 2\beta_1\,\ell n\,\beta_1 + \beta_2\,\ell n\,\beta_2) + (1-Z)\,(a_0\,\ell n\,a_0 + a_1\,\ell n\,a_1)\} \tag{13}$$

Before minimizing the free energy it must be recognized that there exist only 2 independent variables, the 5 probability parameters being interconnected by the constraints (EQUATIONS 5, 11, and 12). The equilibrium conditions then read

$$\mu_a = \mu_g = \frac{1}{L}\left\{\frac{\partial(E-TS)}{\partial a_1} + \frac{\partial(E-TS)}{\partial a_0}\frac{\partial a_0}{\partial a_1} + \frac{\partial(E-TS)}{\partial \beta_0}\frac{\partial \beta_0}{\partial a_1} + \frac{\partial(E-TS)}{\partial \beta_1}\frac{\partial \beta_1}{\partial a_1}\right\} \tag{14}$$

$$0 = \frac{1}{L}\left\{\frac{\partial(E-TS)}{\partial \beta_2} + \frac{\partial(E-TS)}{\partial \beta_0}\frac{\partial \beta_0}{\partial \beta_2} + \frac{\partial(E-TS)}{\partial \beta_1}\frac{\partial \beta_1}{\partial \beta_2}\right\} \tag{15}$$

From EQUATIONS 14 and 15 it is evident that a_1 and β_2 had been selected arbitrarily as the independent variables, and that a_0, β_0, and β_1 are to be expressed in terms of the remaining two.

When the indicated differentiations are carried out, one finds

$$\mu_g = (1-Z)\left[(\epsilon_1 - \epsilon_0) + kT\,(\ell n\, a_1 - \ell n\, a_0)\right] + Z\left[\zeta_1 - \zeta_0 + kT\,(\ell n\, \beta_1 - \ell n\, \beta_0)\right] \tag{16}$$

$$0 = (Z/2)\left[\zeta_0 - 2\zeta_1 + \zeta_2 + kT\,(\ell n\, \beta_0 - 2\ell n\, \beta_1 + \ell n\, \beta_2)\right] \tag{17}$$

To fix the energy levels, set $\epsilon_0 = \zeta_0 = 0$, $\epsilon_1 = \zeta_1 = -\epsilon$, and $\zeta_2 = -2\epsilon + w$, where w is the lateral interaction energy. If then μ_g is replaced by EQUATION 7, EQUATIONS 16 and 17 may be rewritten as

$$(a_0/a_1)^{(Z-1)}\,(\beta_1/\beta_0)^{Z} = K_1 \equiv p/p^0 \tag{18}$$

$$\beta_0\beta_2/\beta_1^2 = C \equiv e^{-w/kT} \tag{19}$$

To solve EQUATIONS 5, 11, 12, 18, and 19, rewrite EQUATION 18 as

$$\beta_1/\beta_0 = Ka_1, \text{ where } K = (K_1 a_0/a_1)^{1/Z},\ a_1 = \frac{a_1}{a_0} \tag{20}$$

then use EQUATION 20 in EQUATION 19 to find

$$\beta_2/\beta_0 = C\,(Ka_1)^2 \tag{21}$$

From EQUATIONS 5, 11, and 12 it is easily established that

$$a_0 = \beta_0 + \beta_1 \quad ; \tag{22}$$

hence we can write

$$\frac{a_1}{a_0} = \frac{\beta_1 + \beta_2}{\beta_0 + \beta_1} = \frac{\beta_1/\beta_0 + \beta_2/\beta_0}{1 + \beta_1/\beta_0} = \frac{Ka_1\,(1 + C\,Ka_1)}{1 + Ka_1} = a_1 \tag{23}$$

which may be converted into a polynomial in K

$$Ca_1K^2 + K\,(1 - a_1) - 1 = 0 \tag{24}$$

with the solution

$$K = \left[a_1 - 1 \pm \sqrt{(1 - a_1)^2 + 4\,Ca_1}\right] \Big/ 2\,Ca_1$$
$$\left\{2\,a_1 - 1 \pm \sqrt{1 + 4a_1\,(1 - a_1)\,(C - 1)}\right\} \Big/ 2\,Ca_1 \tag{25}$$

The negative sign preceding the square root, henceforth replaced by the symbol B, is physically meaningless; thus EQUATION 25 becomes

$$K = (B - 1 + 2a_1)/2a_1C \tag{26}$$

and in view of the definitions for K, K_1, and C, we obtain the final result

$$\frac{p}{p^0} = K_1 = \frac{a_1}{1 - a_1}\left[\frac{B - 1 + 2a_1}{2a_1}\right]^z e^{zw/kT}$$
$$= \frac{\theta}{1 - \theta}\left(\frac{B - 1 + 2\theta}{2\theta}\right)^z e^{zw/kT} \tag{27}$$

which, apart from the absence of the factor Q_s and from a trivial difference in the definitions of w, is the well-known Fowler-Guggenheim adsorption isotherm.[5] The result is quite reasonable, for in regarding the lattice as a superposition of bonds and points, the interaction between atoms adsorbed on nearest neighbor sites can be taken into consideration. In such a case one must recover the equation previously obtained[5] for this specific physical situation; the present treatment in its relative brevity possesses some advantage over the original work.

Extensions of the Theory

Up to this point we have derived previously known adsorption isotherm equations as special cases of a very general approach. Unfortunately, it is not possible to obtain improvements on the prior approximations without recourse to a rather complex formalism that is beyond the scope of this paper. However, a qualitative account of the method and results is instructive.

As before, the first task consists in decomposing the lattice into representative figure arrays. In the case of a triangular lattice, the second-order approximation is based on the triangular subfigure, containing bonds and points as smaller units. The number of members in each array is adjusted so that the entire collection correctly represents the number of triangular units, nearest neighbor pairs, and sites in the lattice of L units. A table of occupation states and energies is constructed similar to TABLE 1, and the energy of the figure arrays as well as their entropy is written out. The resulting free energy is minimized with respect to the independent variables that remain after normalization and consistency equations are set up. The condition $\partial F/\partial a_1 = \mu_g$ represents the isotherm equation; all remaining equations furnish interrelations between the unknowns a_0, a_1, and β_i.

As expected, the mathematical complications increase enormously with successive approximations. In the second-order case, one can still obtain the final result in closed form. The relation between p and θ is found by solution of the quartic

$$K^4 + a_1 K^3 + a_2 K^2 + a_3 K + a_4 = 0 \tag{28}$$

where

$$a_1 = \frac{(2-5\theta)\,C + (2-3\theta)}{C\,(1-2\theta)} \quad ; \quad a_2 = \frac{C+5}{C}$$

$$a_3 = \frac{(3-5\theta)\,C + (1-3\theta)}{C^2\,(1-2\theta)} \quad ; \quad a_4 = \frac{1}{C^2} \tag{29}$$

and where K is given by EQUATION 20 and K_1 by EQUATION 18.

In the third-order approximation to the triangular lattice, the rhombus is considered as the highest subfigure. This case is of special interest because it is possible here to take account of next-nearest neighbor interactions, w^1, along the long diagonals of the rhombus. The HB procedure for this case leads to a system of 18 equations, 6 being nonlinear. Since there seems to be no hope of obtaining closed analytic expressions, numerical methods for finding solutions were developed; these involve extensive machine computation.

Some typical results are assembled in FIGURES 1 and 2 for different

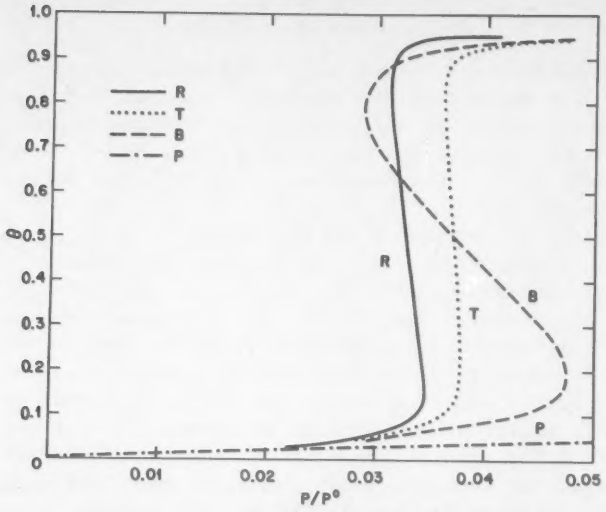

FIGURE 1. Adsorption isotherms calculated for various approximations in order-disorder theory. Case *P:* .Langmuir isotherm; case *B:* Fowler-Guggenheim isotherm; case *C:* triangle (second-order) approximation; case *R:* rhombus (third-order) approximation. $C = 3$, $C' = C^{1/27}$; $Z = 6$. The interactions are attractive.

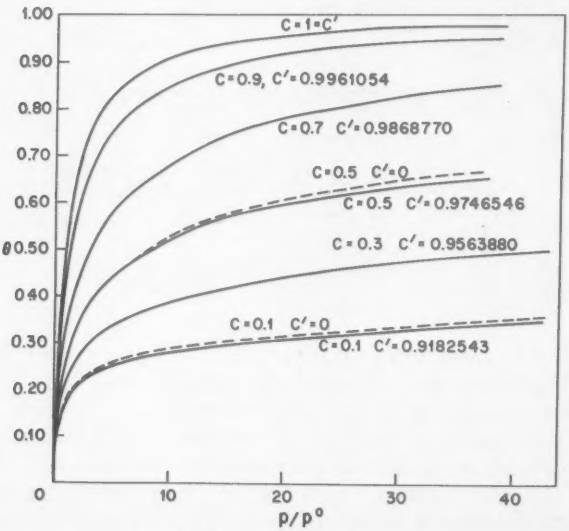

FIGURE 2. Adsorption isotherms for the case of repulsive lateral interactions based on the second- ($C' = 1$) and third-order approximations. The numerical C and C' values are indicated; $Z = 6$.

values of the parameters C and $C' \equiv \exp w'/kT$, and with $Z = 6$. Inspection of FIGURE 1 shows that the Fowler-Guggenheim isotherm (case B) lies far above the Langmuir isotherm (case P) over most of the pressure range considered. This very drastic change is obviously due to the lateral interaction between adsorbed particles. Under conditions assumed in the calculations, w/kT is sufficiently large so that cooperative condensation sets in, as evidenced by the loops in the curve B. The actual isotherm thus exhibits a discontinuity (vertical straight line) passing through the point $\theta = 1/2$. As one passes to the triangle approximation (second order, case T), the loop decreases in size, showing that the extent of cooperative condensation is diminished; this phenomenon is well known [1] and is due to the fact that, as the subfigures increase in size, greater selection of sites becomes available as potential adsorption centers. Thus the energy gain associated with clustering is partially offset by the entropy gain associated with a more random distribution. Finally, in passing to the rhombus approximation (third order, case R) one observes that the loop has increased somewhat in size relative to case T and that the entire isotherm lies above the isotherms T, B, and P. This is a reflection of the next-nearest neighbor interactions. It is seen that this effect is by no means negligible, but that it is not as drastic as the effect associated with the passage from case P to case B.

So far the interactions have been assumed to be attractive; the case of repulsive interactions is summarized in FIGURE 2. It is seen that all curves resemble the Langmuir isotherm ($C = 1 = C'$). They yield a fairly good straight-line Langmuir plot over a reasonable pressure range. As anticipated, the stronger the lateral interactions, the greater is the pressure required to reach a specified value of θ. The effect of the next-nearest neighbor interactions is quite small, as may be seen by comparing the dotted and full curves. In the present case the adsorbed molecules tend to be separated from one another; here the energy and entropy effects work in the same direction. Thus, at intermediate coverage of the surface the lateral interaction effects are not significantly changed when second-nearest neighbor repulsion is taken into account.

Acknowledgment

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EXPERIMENTAL YELLOW FEVER*

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Introduction

Jungle yellow fever,[1a] which during the last fifty years has been confined largely to monkeys living in the forests of South and Central America, is now approaching human habitations in open spaces of the North American continent whence it can be transferred to man and be spread on a potentially significant scale by suitable vectors. From such areas, it could then be introduced to other vulnerable parts of the world by air transport of passengers harboring the virus before clinical symptoms become apparent.

Because of the possibility of this dissemination of yellow fever, it would seem desirable to be able to detect the presence of the virus or its effects in the earliest possible stage of the disease. The clinical diagnosis[1e] during this phase is difficult, but can be confirmed by serologic means, applying the mouse protection test,[1b,2] which takes several days, or by performing a liver needle biopsy. The latter procedure is considered somewhat hazardous in the more advanced stages of the disease; however, the pathological changes in the liver have been regarded as pathognomonic since their description by Councilman[3] more than fifty years ago. The autopsy findings that he described were later confirmed by Klotz and Belt[4a-c] and others,[1c] as well as in experimental yellow fever infections of monkeys by Bearcroft.[5]

The obvious importance of yellow fever as a domestic as well as an international problem prompted the United States Department of Health to suggest a re-evaluation of its biological manifestations by experimental methods and application of new techniques for diagnosis. Accordingly, a study in animals was carried out by a team constituted of members of

*This paper, illustrated with slides, was presented at a meeting of the Section on February 8, 1960.

†Coordinator of this study.

the staff of the United States Army Medical Unit at Fort Detrick and of the Armed Forces Institute of Pathology.

Materials and Methods

The protocol was designed to permit the examination of blood and other selected tissues from experimentally infected animals at 18- to 24-hour intervals in all stages of disease from the time of initiation of the infection until death of the animal was imminent. The host employed was the susceptible *Macaca mulatta* monkey, and the virus the *Asibi* strain. Seed material used was infective monkey plasma.

On the basis of a small pilot study, a virus dose was selected that could be expected to permit survival of at least a portion of the animals for a period of 6 to 7 days following infection. For the final study, a group of 14 monkeys of mixed sex and averaging about 2.5 kg. in weight was chosen. Prior to inoculation, bleedings were obtained for base-line serologic, hematologic, and clinical chemistry examinations. During a 14-day preinoculation period rectal temperatures were taken daily, and base-line chest X rays and EKGs were obtained.

Monkeys were infected intraperitoneally with 10^3 mouse I.C. LD_{50} units of plasma virus. Subsequent to challenge, temperatures were taken rectally at 6-hour intervals, and the animals were closely observed for clinical signs. EKGs were taken on alternate days and chest X rays prior to sacrifice.

Beginning on the morning of the first day postchallenge, 2 animals per day were sacrificed. Nembutal was administered to provide surgical anesthesia, a laparotomy performed, and 2 liver biopsies obtained under direct visualization. Biopsy specimens were processed immediately, one third of 1 specimen being transferred to 1 per cent osmic acid and the remaining two thirds placed in buffered 10 per cent formalin. The second biopsy specimen for fluorescent antibody studies was divided; one half was quick-frozen in liquid nitrogen, and the other half was placed in cold acetone.

After liver biopsies were obtained, the animals were sacrificed by exsanguination. A series of blood films was prepared; a small portion of blood was collected in oxalated tubes, and the remainder allowed to clot for serum. Serum not examined immediately was maintained in the frozen state at –60° C. until tests were performed.

Following exsanguination, a complete autopsy was performed. Multiple tissue imprints were prepared from liver, spleen, and superficial lymph nodes. Blocks of these and other tissues were quick-frozen for virus studies and formalin-fixed for histological examination.

Experimental Results

Virus titrations. Intracerebral titrations of the various tissue suspensions of infected monkeys in young mice showed that virus was not

recoverable in the first 24 hours from 10 per cent or more dilute homogenates of liver, serum, kidney, spleen, lymph node, bone marrow or brain. Twenty-four hours postinfection, liver, serum, and kidney contained increasing amounts of virus, which subsequently dropped significantly 120 hours following inoculation. Virus in lymph nodes, spleen, bone marrow, and brain was demonstrated 48 hours after infection and diminished after 120 hours. In general, liver emulsions consistently showed the greatest virus content,[1d] attaining peak titers on the fourth and fifth days in the order of 10^9 mouse LD_{50} units of virus per gram of tissue. This level of virus was also approached in serum at the same period of time. Results of these studies are given in FIGURE 1.

Serum antigen determinations. It has been shown[6] (J. A. Kerr, personal communication) that the serum of yellow fever-infected monkeys may possess complement-fixing antigens during the acute phase of the disease. High titers of chick cell hemagglutinin that could be inhibited by yellow fever antiserum were demonstrated by Porterfield.[7] These findings were readily confirmed with infective monkey serum obtained at the

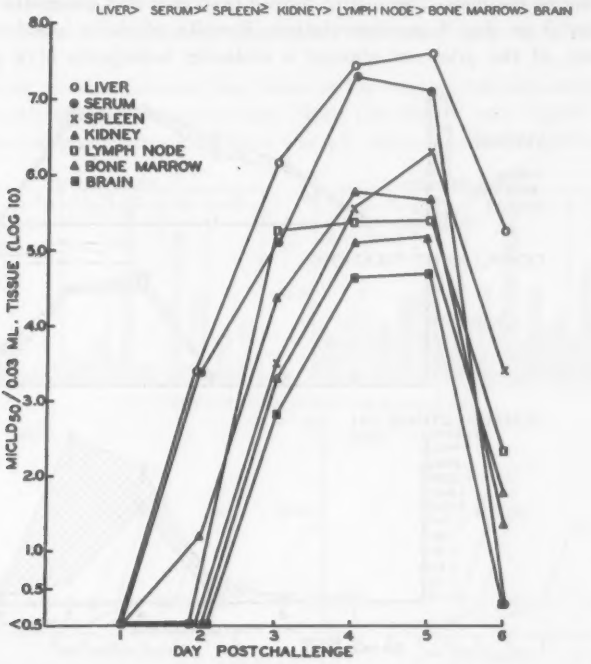

FIGURE 1. Yellow fever virus content in tissues of monkeys sacrificed at given intervals following intraperitoneal inoculation with 1.0×10^3 mouse intracerebral doses of *Asibi* strain. United States Army photograph.

height of the experimental disease. Normal complement (C'4 fraction) was inactivated with NH_4OH. Normal serum hemagglutinins and hemagglutination inhibitors were removed by treatment of the infective sera with protamine sulfate, followed by adsorption with chick cells in borate saline at *p*H 9.0. Results of the tests are summarized in FIGURE 2.

Paired monkeys sacrificed on days 1 through 6 showed a remarkable uniformity in response as measured by viremia and complement-fixing antigen. Specimens from 6 animals tested on days 4 and 5 after inoculation, on the first or second day of fever, showed viremia levels varying between $10^{6.6}$ and $10^{7.4}$ mouse I.C. LD_{50} units, or a sixfold difference between highest and lowest titers obtained. Of these same sera, 4 showed complement-fixing antigen titers of 1:256 and the other two 1:512, or twofold variation.

Hemagglutinin levels were found to vary far more widely, from 1:10 to 1:10,240 in the face of closely similar serum virus titers. It appears that correlation between serum virus levels and titer of hemagglutinins is poor (FIGURE 2).

Clinical findings. The initial febrile response was generally observed on day 3 or day 4 postinoculation. Results of daily blood counts in animals of the pilot run showed a moderate leukopenia at a challenge

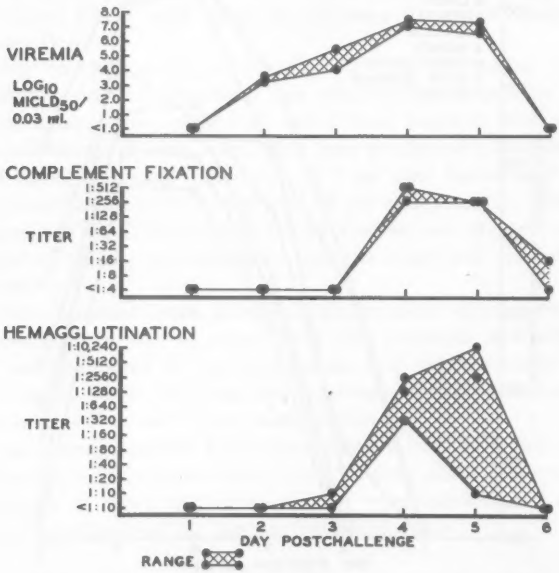

FIGURE 2. Virus, complement-fixing antigen, and chick cell hemagglutinin in sera of monkeys sacrificed at daily intervals following inoculation with *Asibi* virus (10^3 $MICLD_{50}$). United States Army photograph.

dose of 10^3 $MICLD_{50}$. With the onset of fever, total lymphocytes decreased, while myeloid cells showed an increase, together with a shift to the left. In animals receiving larger challenge doses of virus, a similar but much more marked series of hematological changes occurred. Prior to death of the animals, some of the white blood counts rose to a higher level than could be explained by hemoconcentration, and showed a predominance of neutrophilic polymorphonuclear leukocytes.

Many of the premyelocytes, myelocytes, and polymorphonuclear leukocytes in bone marrow preparations exhibited a peculiar vacuolization of their cytoplasm during the later phases of yellow fever.

Serial daily electrocardiograms and roentgen chest films made at the time of sacrifice were within normal limits.

Clinical chemistry determinations of blood urea nitrogen, cholesterol, phosphorus, and glucose levels showed no significant deviation from the base-line levels. Cephalin flocculation and thymol turbidity values likewise showed no consistent deviation.

Animals sacrificed on days 4, 5, and 6 postinoculation showed a modest increase in total bilirubin without change in the direct reading portion. Rises in alkaline phosphatase were also seen at this time (FIGURE 3).

The most marked changes were seen in the serum glutamic-oxalacetic and glutamic-pyruvic transaminases. Both the SGO-T and SGP-T values increased precipitously on days 4 and 5, when values of 1,000 u./ml.

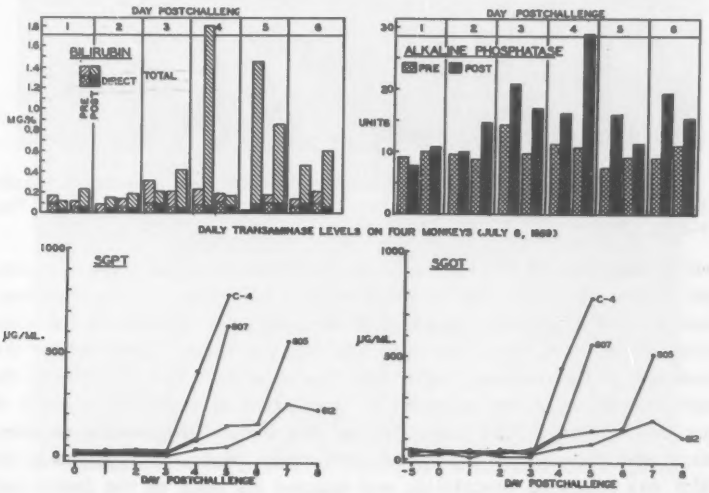

FIGURE 3. Serum transaminase, bilirubin, and alkaline phosphatase determinations in sera of monkeys experimentally infected with *Asibi* virus. United States Army photograph.

were reached. This observation was subsequently confirmed in an additional group of monkeys when serial daily blood samples were drawn from the same animals (FIGURE 3).

Histopathological studies. The only significant finding seen in the liver tissue obtained by needle biopsy up to the fourth day following experimental infection was an eosinophilic necrosis of Kupffer's cells, sometimes accompanied by the presence of a few poorly preserved neutrophilic leukocytes in the neighborhood of the reticuloendothelial cells (FIGURES 4,5). No evidence of degeneration or necrosis of liver cells

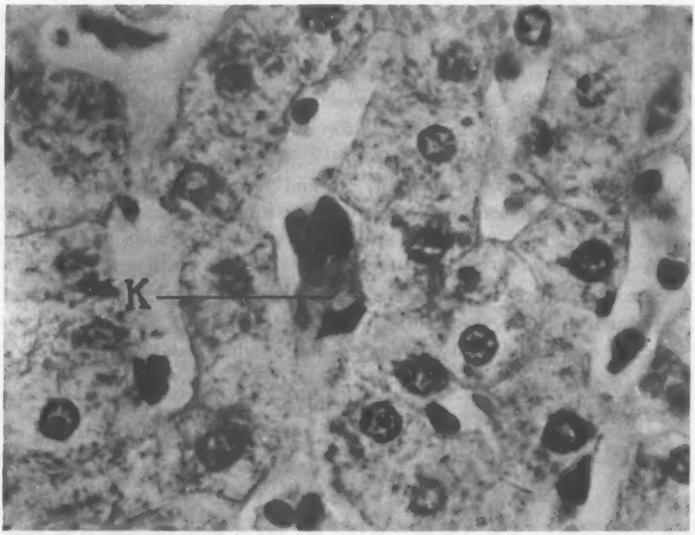

FIGURE 4. Liver needle biopsy, monkey, 24 hours after inoculation. Acidophilic hyaline necrosis of Kupffer's cell (K). Hematoxylin and eosin. AFIP Neg. 59-3772. ×900.

was present at this time. Some of the reticuloendothelial cells of spleen and lymph nodes, as well as glial cells of the central nervous system, also showed acidophilic necrosis of the cytoplasm. Studies of the biopsies by means of the fluorescent antibody technique[8] demonstrated the presence of fluorescence within the Kupffer's cells (FIGURES 6,7). No such fluorescence was apparent in liver cells or parenchymal cells of any other organ at this time. Gradual but rapidly progressing degeneration and necrosis of individual liver cells became evident after the third day following inoculation and reached its peak on the fourth and fifth days (FIGURE 8). At this time fluorescence was again present in many Kupffer's cells and liver cells. Animals surviving the sixth day presented more focalized lesions of a granulomatous nature (FIGURE 9).

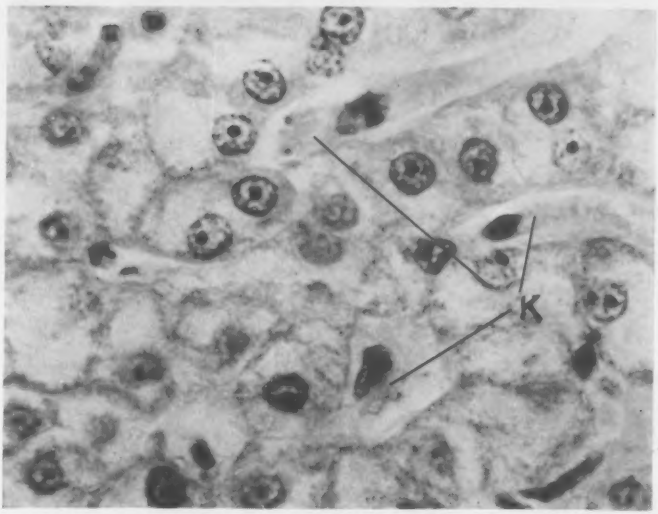

FIGURE 5. Liver needle biopsy, monkey, 48 hours after inoculation. Acidophilic hyaline necrosis of several Kupffer's cells (K). Hematoxylin and eosin. AFIP Neg. 59-3784. ×900.

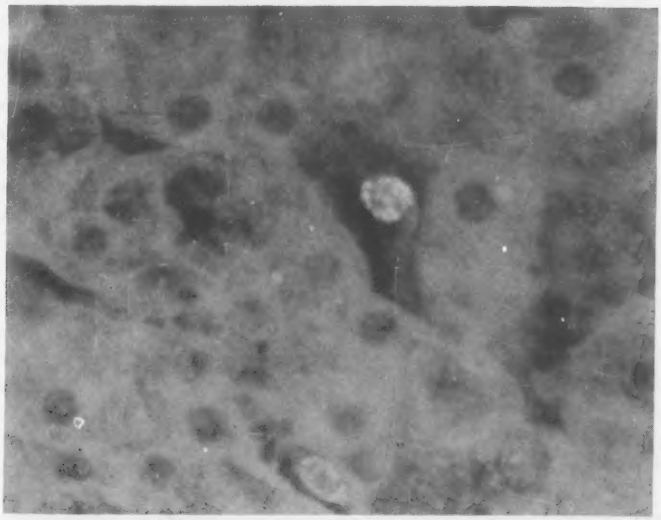

FIGURE 6. Liver needle biopsy, monkey, 19 hours after inoculation. Fluorescent antibody technique. Fluorescence in occasional Kupffer's cells. AFIP Acc 912238 (M755), ×350.

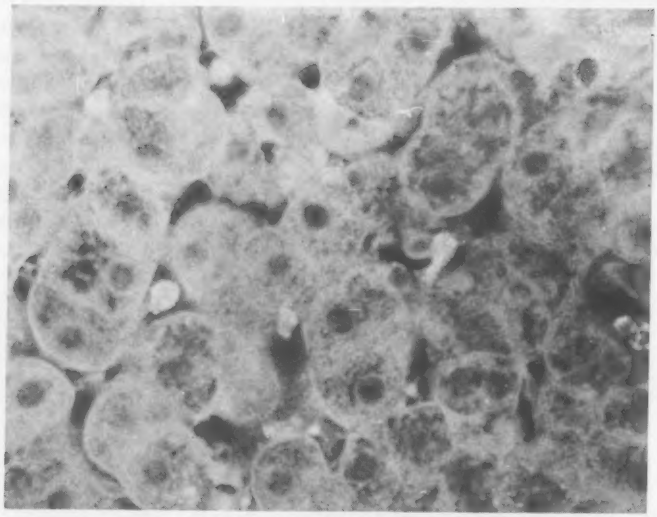

FIGURE 7. Liver needle biopsy, monkey, 72 hours after inoculation. Fluorescent antibody technique. Fluorescence in many Kupffer's cells. AFIP Acc 921238 (M1095). ×260.

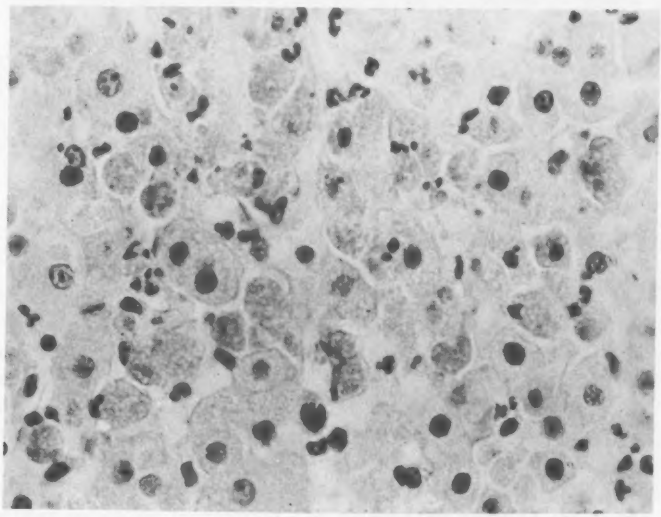

FIGURE 8. Liver needle biopsy, monkey, 92 hours after inoculation. Extensive hepatic necrosis with numerous Councilman bodies. Hematoxylin and eosin. AFIP Neg. 59-3801. ×350.

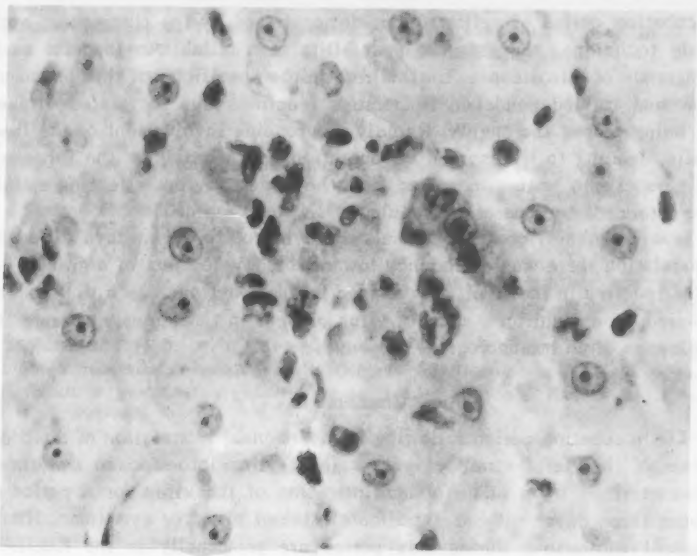

FIGURE 9. Liver needle biopsy, monkey, 144 hours after inoculation. Granulomatous reaction. Few Councilman bodies. Hematoxylin and eosin. AFIP Neg. 59-3802. ×900.

Intranuclear inclusions in liver cells were not a constant feature during the early phase of yellow fever, but occurred more frequently during the later phases of the disease. Electron microscopic studies of liver biopsies showed closely packed granular matter, probably representing aggregates of virus, in Kupffer's cells during the early phases of yellow fever. Extensive alterations of the liver cells with fusing and destruction of mitochondria were seen in later phases.

Summary

Virus was initially detected on day 2 postchallenge and increased thereafter in all tissues examined through day 5. The content was always highest in liver, followed closely by serum. Shortly after or coincident with the onset of fever, by day 4, complement-fixing antigens and chick cell agglutinins were demonstrable in serum. Neither these antigens nor free virus could be demonstrated in the sera of animals sacrificed on day 6, and virus levels in other tissues showed a significant decrease.

Characteristic acidophilic hyaline necrosis was observed in the reticuloendothelial cells, particularly in the Kupffer's cells, during the first three days following inoculation of yellow fever virus, in the absence of significant clinical disease and laboratory evidence of hepatic dysfunction. The presence of fluorescence in Kupffer's cells during the

incubation period of yellow fever, demonstrated by the fluorescent antibody technique, suggests the possibility of a reliable method for early diagnosis of this disease; for this reason the specificity of this phenomenon and its independence from cross reactions due to related viruses is being probed thoroughly. Rapidly progressing involvement of the liver cells, leading to widespread degeneration and necrosis in the formation of Councilman bodies, occurred from the fourth day on, affecting mainly the intermediate zone of the lobules.

In a few observations of animals surviving up to seven days following inoculation there was a tendency toward the development of a granulomatous reaction in the liver; this altered tissue reaction, which was accompanied by a significant drop of virus present in the serum, suggests the influence of an immunological response.

Conclusions

The incubation period following intraperitoneal inoculation of a modest dose of the *Asibi* strain of yellow fever virus into *Macaca mulatta* is characterized by a stage of multiplication of the virus for a period of about three days, without significant clinical signs or symptoms. Histological alterations during this period are principally in the Kupffer's cells of the liver, after which an explosive change in the parenchymal liver cells occurs.

The diagnosis of yellow fever in the experimental animal can be suspected by the presence of acidophilic degeneration in Kupffer's cells of the liver within twenty-four hours after inoculation. The possible confirmatory demonstration by the fluorescent antibody technique of a specific antigen exhibiting fluorescence in reticuloendothelial cells must be probed thoroughly by further studies.

This sequence of findings in the monkey is sufficient to suggest that a liver biopsy may be warranted as a diagnostic procedure in humans suspect of yellow fever who show an increase in temperature and elevated serum transaminases.

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DIVISION OF INSTRUMENTATION

THE INTERDEPENDENCE BETWEEN INSTRUMENTAL AND CHEMICAL ANALYTICAL APPROACHES*

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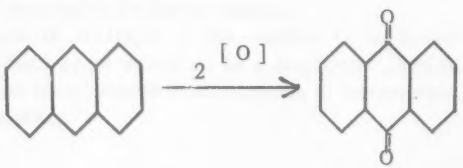
In the actual practice of analytical chemistry, one generally encounters a separation between chemical analysis and instrumental analysis. This separation is due, I feel, to an incompatibility in the required aptitudes. A chemical analyst (or chemist) generally has little aptitude, hence little interest, for instrumental analysis. The converse is also true: the instrumental analysts will generally avoid "wet" methods.

This segregation of analytical approaches is therefore a natural one and thus unavoidable. However, the problem is not insurmountable. If each type of analyst realizes what the other can do for him, he can call on his colleague to do the necessary work which he himself cannot do. It is the purpose of this paper to point out the interdependence between instrumental and chemical analytical approaches so that the complete analytical "tool kit" may be used, each tool having a function it can perform better than any other. Since each approach also has weak points, there are times when it should not be used. Thus, in the solution of analytical problems, it is not surprising to see combinations of tools being used to arrive at the final conclusion.

As an example, the case of an analytical system used to monitor the following reaction can be cited:



This system was complicated by the fact that phthalic and maleic anhydrides and their corresponding acids were formed during the reaction along with the anthraquinone. Also, the anthracene used was a crude material containing significant quantities of phenanthrene and carbazole. Thus, the reaction mixture containing all these components was quite complex. The final analytical system devised for the mixture is shown in TABLE 1.

The above system shows the interdependence between the chemical and instrumental methods in completing the job to be done.

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TABLE 1

Component	*Analytical approach used*
Anthraquinone	Polarography
Anthracene	Ultraviolet absorption (correction for anthraquinone used)
Phenanthrene	Ultraviolet absorption
Total anhydrides	Wet chemical methods
Total carboxylic acids	Wet chemical methods
Carbazole	Nitrogen analysis

We can see this interdependence even in individual approaches. For example, in infrared absorption analysis, it is often difficult to distinguish –NH– from –OH absorption bands. However, by applying a chemical test (sodium fusion), the absence or presence of nitrogen can be established. For quantitative discrimination between –OH and –NH– there are chemical methods that are specific for each group. Thus, chemical methods have filled a gap in infrared absorption technology.

Conversely, chemical methods frequently cannot distinguish position isomers, but infrared absorption analysis often can make the distinction.

TABLE 2 shows the general analytical tool kit, highlighting the general strong and weak points of each method. It is well to state that all ratings in the table are general and that these might not apply to specific instances. An approach may be *generally* weak in a certain category, but in a selected chemical situation it may well be strong.

It may be seen from TABLE 2 that there are preferred methods for handling given situations, that is, spectrographic emission for trace elements; polarography, ultraviolet absorption, or colorimetric analysis for trace organic materials; and freezing point assay for assays in the range of high purity. Although admittedly crude, this table gives some idea of the inability of any approach to stand alone and shows that utilization of the tool kit as a whole covers the range of analytical situations fairly completely when one considers accomplishment of the job, conclusiveness of results, and time required for analysis.

We have now seen the intertwined usage of chemical and instrumental methods. However, the interdependence of chemical and instrumental methods goes beyond this. We can extend the range of instrumental approaches by chemical alteration of the sample. Thus, even though an instrumental approach cannot be applied to the sample as received, it can be made to operate on the chemically altered sample.

One well-known example of this is colorimetric analysis where the material being measured is itself colorless, but a reagent renders it

TABLE 2

GENERAL SENSITIVITY

Analytical approach	*General specificity*	*Trace quantity range*	*Medium range*	*High purity range*
Elementary (organic) wet methods	Excellent for elements, poor for compounds	Generally poor, good for some elements	Good	Good
Elementary (inorganic) wet methods	Excellent for elements, poor for compounds	Generally poor	Good	Good
Organic wet methods	Generally good	Poor to fair	Excellent	Fair
Spectrographic	Excellent	Excellent	Fair to poor	Poor
X-ray diffraction	Excellent	Poor	Good	Good
X-ray fluorescence	Excellent	Poor	Good (1 to 10%) Fair (> 10%)	Poor
Polarography	Poor to fair	Excellent	Fair	Poor
Infrared absorption	Fair to good	Poor	Excellent	Fair
Ultraviolet absorption	Poor to fair	Excellent	Fair	Poor
Visible absorption	Poor to fair	Good	Good	Poor to fair
Vapor chromatography	Good	Poor to fair	Excellent	Poor to fair
Freezing point and freezing curve	Poor	Poor	Poor	Excellent
Mass spectrometry	Good	Poor to fair	Good	Poor to fair
Microscopy*	Fair to good	—	—	—

*Microscopy is an approach rather by itself; the headings of the table do not apply.

GENERAL SENSITIVITY

General precision and accuracy	*Qualitative use*	*Quantitative use*	*General time of analysis*	*General sample limitations*
Good	Good	Good for elements, poor for compounds	Fair to good	None
Good	Good	Good for elements	Generally long	None
Poor to excellent, depending on ranges	Poor to fair	Poor to excellent, depending on ranges	Generally long	None
Good in trace ranges, poor in other ranges	Excellent	Good in trace ranges, poor in higher ranges	Shorter than wet methods (after calibration)	None
Poor for quantitative use	Excellent	Fair to poor	Good	Components must be crystalline
Poor to good, depending on range	Excellent	Poor to good, depending on range	Shorter than wet methods	Rigid sample preparation
Poor to fair	Poor	Excellent in trace range, poor to fair in other ranges	Fair to good	Component must be electrically oxidizable or reducible
Good	Good	Good except in trace and high purity ranges	Good	Component must absorb IR radiation
Poor to excellent, depending on range	Poor to fair	Excellent in trace ranges, poor to fair in other ranges	Good	Component must absorb UV radiation
Good	Poor	Good in lower ranges, poor in higher ranges	Poor to good, depending on method	Component must absorb visible radiation
Good	Fair to good	Excellent	Short	Must be volatile within operating limits of the instruments
Excellent in high purity, poor in the other ranges	Excellent for proof of purity of unknown, but poor for identification	Excellent for high purity range but poor in other ranges	Fair	Sample must be crystallizable
Good, except at extreme ranges	Good	Good, except at extreme ranges	Fair, once equipment is operating and calibrated	Components must be volatile within the limits of the instrument
—	Good	Poor	—	Sample must have visible characteristic parameters

colored so that it can then be measured instrumentally.

Another example can be cited from the anthraquinone system discussed above. Polarographic analysis for determining anthraquinone was not possible on the sample as received; the anhydrides interfered. However, by adding lithium hydroxide, the anhydrides were converted to lithium salts, which did not interfere.

Still another example can be supplied in the use of vapor chromatography. This approach achieves separation of volatile materials by passage through a suitably packed column. However, it sometimes occurs that the components of certain samples are not sufficiently volatile for the use of this approach. In these cases it is often possible to achieve the necessary volatility by converting the sample components to other forms. For example, fatty acids cannot be separated by some of the existing vapor fractometers. However, if these acids are converted to the corresponding methyl esters, separation is made possible.

The above examples should suffice to illustrate the interdependence between the chemical and instrumental approaches. Each analyst should be informed on the full analytical tool kit even though he cannot be expected himself to use each tool directly. He should, however, know when to call on specialists in other areas to bolster the weaknesses in his own area of specialty. This approach leads, not only to a broader application of analysis to chemical systems, but also to the most conclusive results in the shortest time.

DIVISION OF NUCLEAR PHYSICS

SLOW NEUTRON INTERACTIONS WITH MATTER*

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At sufficiently high energies, the scattering of a neutron by a system of nuclei is independent both of the forces that bind the nuclei together and of their thermal motion. As the neutron energy decreases and approaches the order of magnitude of the chemical binding energy of a nucleus (that is, ~1 ev), these binding forces will tend to increase the scattering cross section. This result can be seen in the following way. The scattering in the forward direction and through small angles is not appreciably altered by the binding forces, since the struck nucleus experiences only a small recoil, whereas the backward scattering is increased owing to the apparent increase in the scatterer's mass as a result of the binding. Thus the total scattering cross section is increased. As an illustration of this effect, we may consider the scattering of a neutron by a bound proton. It was first shown by Fermi[1] that the zero energy cross section of the bound proton is four times as large as the free neutron-proton cross section. In general, for neutron scattering by a target of atomic mass number A, the bound cross section is larger by the factor $(A+1)^2/A^2$. This increase is known as the "reduced mass" effect, since it can be derived from considerations of the reduced mass of the system.

Fermi's derivation is based on the use of his so-called "pseudopotential," which is a delta function interaction whose magnitude is adjusted to give the correct scattering length a:

$$V(r_n - r) = \frac{2\pi\hbar^2 a}{\mu}\,\delta(r_n - r)$$

where V is the Fermi pseudopotential, a is the scattering length and μ is the reduced mass of the system. Hence

$$a = \frac{\mu}{2\pi\hbar^2}\int V(r)\,dr$$

and since $\sigma \sim a^2$, we have $\sigma \sim \mu^2$. Now $\mu_{free} = \frac{A}{A+1}$ while $\mu_{bound} = 1$, both measured in neutron masses. Thus

$$\frac{\sigma_{bound}}{\sigma_{free}} = \left(\frac{A+1}{A}\right)^2.$$

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Another interesting example of the effect of chemical binding on slow neutron scattering that can be solved analytically is that of scattering by a proton bound harmonically to a center of force. If the neutron energy is less than $\hbar\omega$, where ω is the proton oscillator frequency, the scattering is elastic, with a cross section given by

$$\sigma_{\text{elastic}}(E) = 4\pi a^2 \frac{1-e^{-4E/\hbar\omega}}{E/\hbar\omega}$$

from which

$$\sigma_{\text{elastic}}(0) = 16\pi a^2 = 4\sigma_{\text{free}}.$$

In the above it has been assumed that the proton is in its ground vibrational state.

When E, the neutron energy, is larger than $\hbar\omega$, inelastic scattering becomes possible. The total scattering cross section is then a sum of contributions corresponding to the various possible final states of the proton. In the limit $E \to \infty$, the cross section is calculated to be $4\pi a^2$, the free particle value, as is to be expected. The asymptotic behavior of the cross section as E becomes very large has been investigated by Placzek[2] and by Wick,[3] in the general case of a scatterer of arbitrary mass A. It is found that when $A > 1$, the cross section executes oscillations in its approach to the free particle value, but that these oscillations tend to decay as $E \to \infty$. An interesting phenomenon associated with the case $A = 1$ was discovered by Placzek. He found that the oscillation persists even at the very highest energies. This effect has been examined in some detail by Wick.[3]

Recent developments in the field of slow neutron scattering have included an application of the very powerful formalism developed by Zemach and Glauber[4] to the problem of scattering by polyatomic molecules[5] and by liquid water (M. S. Nelkin, unpublished data).

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DIVISION OF PSYCHOLOGY

AN EXPERIMENTAL ANALYSIS OF THE ROLE OF CONTEXT IN VERBAL BEHAVIOR*

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In a series of experiments (see Gonzalez and Cofer, 1958, for summary), the effects of modifiers on words were explored by investigation of clustering in free recall. The clustering phenomenon, first described by Bousfield (1953), is studied in the following way. A set of words drawn from four categories is developed. The words are then presented in a random order to subjects who recall them in the order in which the words occur to them. Characteristically, the recalls show nonrandom sequences of words from the same categories, that is, the words in recall tend to cluster in categories to an extent greater than chance expectation.

In his work, Bousfield has used chiefly lists of nouns. In order to explore modifier effects, Gonzalez and Cofer presented their subjects with randomized lists of *pairs* of words. The pairs consisted of a modifier and of a modified word, such as adjective-noun pairs, adverb-verb pairs and the like. The investigators showed a number of effects:

(1) *Specificity effect.* When adjectives were chosen to modify nouns so that a given adjective pertained to only one noun in the entire list and when no two adjectives belonged to the same category, clustering of the nouns in noun-recall was diminished to chance levels.

(2) *Inappropriate modification effects.* Clustering of nouns was considerably impaired when the adjectives that modified them were inappropriate, for example, starched bulldog or leafy lawyer.

(3) *Mediation effects.* When a list of nonclustering (uncategorized) nouns was modified by adjectives that fell into categories and that, presented alone, clustered, the nouns, when recalled and scored for clustering on the basis of the adjectives that modified them, showed significant clustering, that is, nonclustering nouns clustered through the categories of the adjectives that modified them. In another experiment, similarly, adjectives were found to cluster in terms of the categories of the nouns they modified. A control experiment showed that similar effects could be obtained when the normal word order was reversed, that is, from adjective-noun to noun-adjective.

(4) *Mediated conflict effects.* Lists were developed of categorized and clustering adjectives and clustering nouns. When adjectives from a given

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category modified nouns from all categories, clustering was impaired.

(5) *Mediated facilitation effects.* This involved setting all the adjectives of one category as modifiers of nouns from only one category. Clustering was enhanced in this case.

(6) *Adverb-verb effects.* Somewhat similar effects to those found for nouns and adjectives were found for adverb-verb and for verb-adverb pairs.

These rather striking effects of modifiers in complex clustering situations require further exploration if they are to be understood. Bousfield *et al.* (1958) have recently shown, in unmodified word lists, that clustering is affected by associative factors. They compared clustering in lists in which the words were frequent associates of the category names with clustering in which the words were infrequent associates of the category names. The former lists showed much more clustering than the latter.

Gonzalez and Cofer (1958) suggested that their results might not readily be based on simple associative relationships and implied that mediating processes perhaps involving meaning might be involved. Before such an interpretation can be justified, however, it is necessary to explore the possibility that associative relations might account for the results Gonzalez and Cofer obtained. This paper reports data that bear on this point.

Method

Six association tests that sampled pairs of words from various conditions were constructed. These pairs of words, which Gonzales and Cofer had used, included single word stimuli as well. The six tests developed included the following materials.

Test 1. Twenty-six word pairs, of which 12 adjective-noun pairs were drawn from the 40 pairs used by Gonzalez and Cofer in the facilitation design, of which 8 were adverb-verb pairs, and of which 6 were pairs used in the mediated clustering study, with reversed word order (noun-adjective).

Test 2. Twenty-six pairs, of which 12 were pairs from the mediated conflict condition, 8 were verb-adverb pairs, and 6 were mediated clustering pairs in reverse order.

Test 3. Twenty-four adjective-noun pairs from the mediated clustering study, usual word order.

Test 4. Twenty-four adjective-noun pairs, of which 12 were from the specificity study and 12 involved inappropriate adjective modification of nouns.

Test 5. Fifty-five single words, including nouns, verbs, and adverbs, which figured in pairs in the first 4 tests.

Test 6. Fifty-four single adjectives, which had figured in pairs in other tests.

The association tests were distributed randomly in sections of introductory psychology and in other undergraduate classes in such a way that 50 cases took each of the first 4 tests, and 99 subjects took test 5 and

test 6. No subject took more than 1 association test, so that the total number of subjects involved is 398.

The subjects were instructed to respond quickly to each pair as a unit or to each single word and to write alongside the pair or the single word the first word they thought of other than the stimulus pair or stimulus word. The subjects were asked to work rapidly and to complete all items.

Results

The results of this study will be presented for each of the 6 effects found by Gonzalez and Cofer as outlined in the introduction.

1. *Specificity effect.* This refers to the abolition of clustering in 40 categorized nouns when each noun is modified by an adjective that pertains to the 1 noun only and when the 40 adjectives themselves do not fall into categories. The comparison to be presented here involves the associations given to the adjective-noun pairs and to the nouns alone. In the original noun list, there were 4 categories: leather objects, wooden objects, metal objects, and objects made of fabric. The 3 nouns from the leather object category may serve for illustration, namely, leather, shoe, and wallet. Each of these nouns, presented singly as stimuli for free association, yields a variety of associations, but there is some overlap. Thus, 38 per cent of the responses to leather are the word shoe, which is, of course, one of the other stimulus words. Leather, itself, occurs as a response to shoe (8 per cent) and to wallet (8 per cent). If associative overlap leads to clustering, these responses would account for the clustering observed among these 3 words. The 3 words, when modified by specific adjectives, appeared in the following pairs: imported leather, tight shoe, and stolen wallet. It is noteworthy that the associative overlap when free associations are made to these pairs drops out, and the responses made to the pairs differ considerably from those made to the nouns alone. For example, the 4 most frequent responses to shoe are foot (feet), horn, sole, and leather, whereas the 4 most frequent responses to the pair "tight shoe" are pain, hurt(s), ouch, and uncomfortable.

These findings are typical of the associative effects arising from the introduction of specific modifiers: whatever associative overlap had existed among the original single nouns seemed to have been reduced or eliminated in the responses to the specifically modified nouns. The specificity effect in clustering would seem explicable on the basis of this reduction of associative overlap by the specific modifiers.

2. *Inappropriate modifier effects.* In the original studies by Gonzalez and Cofer, inappropriate modifiers reduced clustering and recall, although for noun recall alone the effects were not as great as they were under the specificity condition. Three of the nouns in the animal category used in that experiment were terrier, collie, and bulldog. Inappropriately modified, these became checkered terrier, cooked collie, and starched bulldog. The

associated responses to the nouns alone overlapped in that dog was a response to each of these stimuli, occurring 49 per cent of the time to terrier, 75 per cent to collie, and 5 per cent to bulldog. This overlap was reduced to some extent in the modified condition. Dog did not occur to the pair, starched bulldog, and its frequency as a response to collie dropped from 75 per cent to 22 per cent for cooked collie. Dog as a response occurred, however, 62 per cent of the time to checkered terrier as compared to 49 per cent to terrier alone. Reduction in associative overlap seemed to characterize the inappropriate modification condition. This result is similar to that found in the specificity condition.

3. *Mediation effects.* There are three cases here: (a) clustering in noncategorized nouns mediated through the categories of the adjectives modifying them; (b) clustering in noncategorized adjectives mediated by the categories of the nouns they modify; and (c) the same as case *a*, but with reversed word order.

Case a. Clustering of nouns mediated by categories of the adjectives that modified them was not very strong, and there are cases in which the associative overlap among the nouns is not increased by the modifier, as one would expect if clustering depends largely on associative overlap. However, there are positive instances. For instance, dress, door, and kettle as stimuli yield no overlapping responses in free association. The combination frayed dress, worn door, and rusty kettle, however, all have in common (and as the most frequent response in all cases) the word old; dirty also occurs to both frayed dress and rusty kettle. Of the four cases from this design studied associatively, this is the only one to yield associative overlap in the modified condition. Such overlap, however, if it occurred for only a few other word combinations, could easily account for the clustering obtained.

Case b. Essentially the same explanation may be found for the clustering of noncategorized adjectives through the categories of the nouns they modified. The adjectives surly, wiry, and gentle, as single stimuli, show no associative overlap. There is associative overlap among surly bulldog, wiry terrier, and gentle collie, mainly involving the response dog. Other instances could be given that would illustrate the same point.

Case c. The response distributions to the pairs in the two word orders are similar, though not identical. The basis for clustering in the uncategorized words in this case appears to be the same as it is for case *a*.

We may conclude that uncategorized words placed in compounds with categorized words will show, in the compounds, associative overlap that they do not show alone. If this associative overlap does attach itself to to the uncategorized words long enough, the clustering in their recalls may perhaps be due to it.

4. *Mediated conflict effects.* In this design, nouns that belong to one category, for example, weapons, are modified by adjectives that belong to all four of the categories into which the adjectives fall. For example,

tunic, jacket, and vest are all articles of clothing, but the adjectives modifying them come from three categories, as illustrated in the pairs decaying tunic, square jacket, and English vest. Clustering of nouns based on the original noun categories was reduced by this type of modification.

Associative data revealed two effects of modification that might lead to this reduction in clustering. The first is simply a marked change in the response hierarchy and a reduction in associative overlap. Thus, unmodified, tunic, jacket, and vest show overlap through the word coat, which accounts for 17 per cent of the responses to tunic, 29 per cent of the responses to jacket, and 13 per cent of the responses to vest. There are also other responses shared between two of these three stimulus words.

Coat as a response, however, does not occur to English vest, and it is reduced to a frequency of 4 per cent in response to decaying tunic and to 6 per cent to square jacket. The response distributions are generally considerably modified by the modifiers, and other bases of overlap are reduced or eliminated. Such effects would, of course, affect the extent of clustering.

The other effect is of a different kind. This consists in a similarity of associations to different pairs produced by the modifier. For example, tunic, pistol, and collie, as single stimuli, show no overlap in their associations. However, they were all modified by adjectives from the "decay" category as shown in the following pairs: decaying tunic, decrepit pistol, and rotting collie. To each of these combinations the word old occurs as a response. If this association carries over to the noun at the time of recall, it would provide a basis for clustering alternative to that provided by the noun categories themselves. This would be an effect similar to that shown in the associations to the pairs used in the mediation studies; however, in the present instance it would provide for conflict between two bases for clustering, and clustering should be reduced, as it is.

5. *Mediated facilitation effects.* In this design all the adjectives from a given category modify nouns from only one category. In the case of tunic, jacket, and vest, for example, the modifiers are all color adjectives, yielding black tunic, brown jacket, and blue vest. Clustering was strongly incremented in this situation.

The associative data in the present case are related much less clearly to the clustering effect than they seem to be in the prior sections. In this, as in all the previous analyses, only a sample of all the items used by Gonzalez and Cofer was studied associatively. In the present instance the degree of the mediated facilitation effect in clustering does not seem to be fully accounted for by the associative data. It may well be, however, that the sample of words available for associative study represented a poor choice.

The two best cases in the present instance are provided by bulldog,

terrier, and dog (modified as square bulldog, short terrier, and round collie) and by cannon, dagger, and pistol (modified as English cannon, Japanese dagger, and Greek pistol). While bulldog, terrier, and collie all elicit dog as a response in free association, this response has a minor frequency (5 per cent) to bulldog. Under the modified condition (square bulldog) this response becomes the most frequent response (20 per cent) and it remains so to short terrier and round collie (52 per cent and 70 per cent, respectively). This change, however, seems to have nothing specifically to do with the adjective category.

Cannon and pistol elicit gun as a common associated response (22 per cent and 21 per cent, respectively). This response increases to 48 per cent in the case of Greek pistol, remaining the same (22 per cent) to English cannon. Dagger and Japanese dagger have no overlap with the other stimuli in either the modified or the unmodified case. Again, the adjective category seems unrelated as such to the change in response distributions indicated, and it is difficult to see how clustering would be much affected by the kinds of changes that did occur.

Tunic, jacket, and vest were modified by color adjectives (black, brown, and blue, respectively). The degree of associative overlap was decreased for the compounds as compared to the single stimuli. It would appear that mediated facilitation is a difficult case to explain on the basis of the available associative data. Unfortunately, this negative conclusion is difficult to accept without considerably more complete data than those available.

6. *Adverb-verb effects.* Gonzalez and Cofer (1958) showed that categorized adverbs alone and categorized verbs alone would cluster. However, they obtained neither a facilitation for verb-adverb pairs (when adverbs were recalled) nor a disruption of clustering. On the other hand adverb-verb pairs (verb recall) showed a decrement in clustering of the verbs as compared to the control condition. In the present study, a sample of verb-adverb and adverb-verb pairs as well as of unmodified verbs and adverbs was used as stimuli for associations. There were only two instances from each category, allowing for a minimum of comparison. The small amount of associative overlap shown in these instances (in both single stimuli and compound stimuli) is consistent with some clustering under both conditions.

Summary

In general, the associative data reported here seem to be consistent with the general idea that the complex clustering effects reported by Gonzalez and Cofer are probably attributable to shifts in distributions of associated responses as a consequence of various arrangements of modifiers. The major exception to this statement was found in the case of mediated facilitation.

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DIVISION OF BIOCHEMISTRY

TUMORS AS UNIQUE BIOLOGICAL TOOLS IN BASIC BIOCHEMICAL STUDIES*

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Scientists and physicians doing cancer research must subscribe, consciously or otherwise, to the dictum that tumors are biochemically different from their parent normal tissues. Although we use the words cancer and tumors as collective terms, the experimental differences that are accumulating to distinguish tumors also from each other are proceeding in the direction of making biochemical individuals of tumors even within histological catagories.

This information carries with it both hopeful and disheartening implications: hopeful, in the prospect of furnishing more exploitable characteristics; discouraging, in reducing the likelihood of finding a successful panacea covering a wide spectrum of malignancies. One can undermine the discouragement by clinging to the analogy of the broad bacterial coverage of some of the antibiotics. The only difficulty with this is that invading foreign bacteria are not endogenous cancer cells subtly derived from the host.

However, if we can accept the essential premise that cancer cells are different from normal cells, what exploitable possibilities exist? For the purpose of this report we shall assume that the directly exploitable contents of a cancer cell consist of: (1) the original substrates delivered to the cell; (2) the enzymes that catalyze the reactions; and (3) the metabolic products of this intracellular biochemistry. To avoid confusion in my subsequent use of the term metabolite, I distinguish between the exogenous or endogenous substrates of the cell (that are ordinarily the components exploited through metabolic analogues), as compared with the secondary intermediates or terminal metabolites that represent the more specialized and unique products of a given cell type. The latter are synthesized within the cell and therefore would be expected to be more definitive in characterizing a cell than the primary raw materials that all tissues receive. This is related to our thesis, since it is the blocked intermediates or terminal products that tend to accumulate in the cell that may lend themselves to therapeutic exploitation.

A part of the rationale of the metabolic analogue is, of course, the incorporation of a masquerading pseudo unit into the basic cellular synthesis, causing a subsequent embarrassment in the production line.

* This paper, illustrated with slides, was presented at a meeting of the Division on February 23, 1960.

A formidable difficulty is that the pseudo substrate is also eligible and available for all cells, normal and malignant alike. The rationale covering this objection is that the pseudo units will be incorporated more effectively into the rapidly growing cancer cells. The success of this approach has been encouraging but limited. Its present rather frustrating status was summarized in a recent seminar reviewing the problem of resistance to cancer chemotherapy for which one of the unhappy titles was "Resistance to Antimetabolites." These expressions of pessimism for the future of the metabolic analogue attack on cancer, as now employed, should be clearly identified as personal and not representing the unanimous opinion of my institute, since I suspect that a number of my "analogue" colleagues would take issue with me. Nevertheless, astute cancer cells have given ample evidence that they are quite capable of adapting to the deprivation or embarrassments of an analogue block. Moreover, the history of biological evolution is the story of the success of cells in adapting to blocks, deprivations, and other impositions.

In regard to other theoretical prospects, I offer here some tentative thoughts stemming from our data bearing on the other two cellular components: the intermediate or terminal metabolites and the associated enzymes.

FIGURE 1 is the familiar diagram of metabolic sequence in cells, indicating the probable consequences of an imposed or natural block

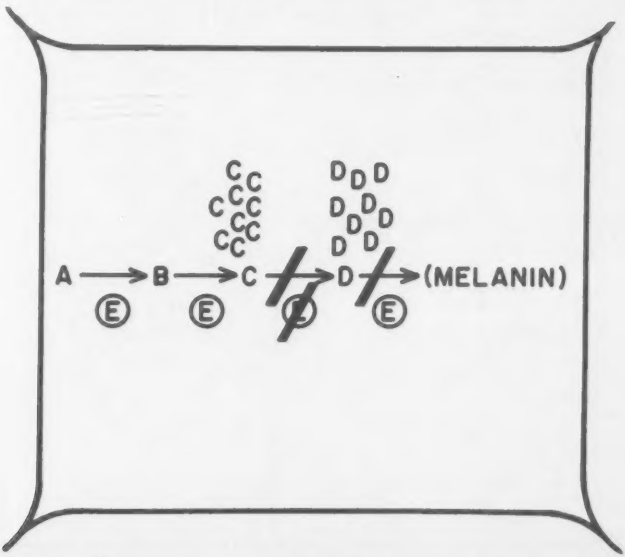

FIGURE 1. Metabolic sequence symbolism showing accumulation of components due to enzyme or polymer (melanin) block.

at any given point. We may be dealing with this phenomenon in the case of our melanoma model, as well as with some other tumor types. The melanoma would seem to be a good candidate for studies of metabolite and enzyme exploitability since it is obvious to the naked eye that there must be exceptional metabolic pathways in this pigmented tumor. Apparently, one of the metabolic missteps in this malignancy is the conversion of the ubiquitous and normal amino acid, tyrosine, to a diphenol, probably dihydroxyphenylalanine (dopa).

FIGURE 2 illustrates the usual textbook pathways for tyrosine in normal cells as compared with its fate in the pigmented melanoma. Whereas tyrosine, as normally handled by the cell, is converted to beneficial physiological products such as thyroxine and adrenaline, it is led into the paths of malignancy when it enters a melanoma cell. I stress the fact that no implication is intended that this melanin-producing pathway is the cause of the malignancy. It is significant primarily as an individual expression of the unique metabolism of this particular tumor. Although dopa and its sequential products may also occur in certain normal pigment-producing cells, there is reason to believe that their existence is ephemeral and the quantities limited. In contrast, the pigmented melanoma cell appears to pile up dopa, or dopalike products, in addition to melanin, as illustrated by the following data.

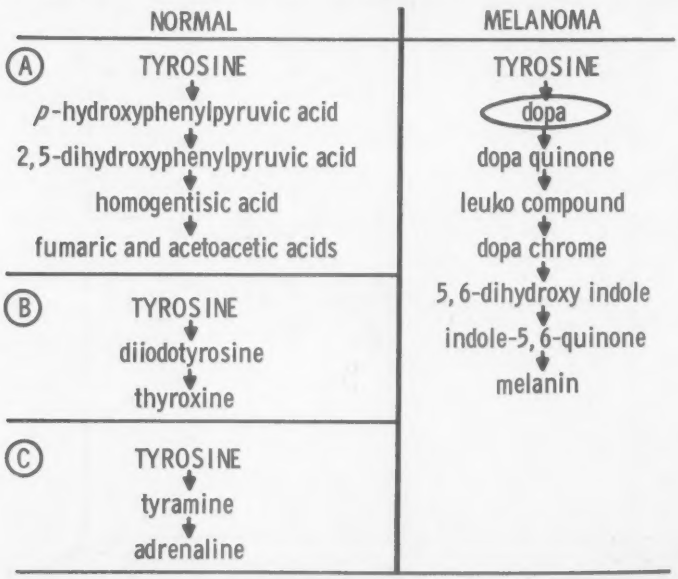

FIGURE 2. Comparison of tyrosine metabolic pathways in normal and in melanoma cells.

It was observed that, when p-phenylenediamine (PPDA) was added to melanoma extracts as a cytochrome c reductant in the course of cytochrome oxidase studies, a nonenzymic oxygen uptake took place.[1] This occurred only in melanomas and was absent in normal tissues and in other tumor types tested. This phenomenon was eventually suspected to be due to one of the metabolites in the melanoma tissue.[2]

FIGURE 3 illustrates the presumed reaction *in vitro*. There are no tissues or enzymes present in this experiment, only the two buffered chemicals PPDA and dopa. For this presentation, these two compounds and their reaction will constitute one of our experimental models.

Testing of various biological materials has demonstrated the presence of PPDA-reacting substances in melanoma tissue, blood plasma, and in the urine of established pigmented melanoma patients.[3] For reasons of simplicity and partial ignorance, we have assumed that the reacting substance in these biological materials is dopa or a dopalike compound.

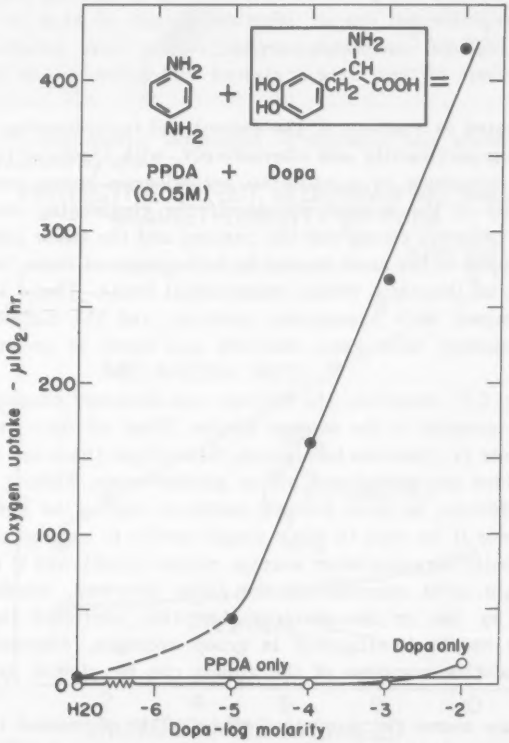

FIGURE 3. Synergistic oxygen-consuming, pigment-producing reaction *in vitro* between dihydroxyphenylalanine (dopa) and p-phenylenediamine (PPDA).

However, dopa quinone and dopa amine will also react with PPDA, and these related substances may also be present in melanoma tissue and fluids.

The PPDA-dopa reaction has an optimum oxygen consumption with a molecular ratio of about 1:1 or 2:1, and it produces a dark brown pigment that is thought to be a polymer.[3]

Following the establishment of an *in vitro* reaction between an accepted tumor metabolite and an exogenous compound, the obvious question was whether there would be any measurable biological consequences if such a drug were injected into tumor-bearing animals. Chemotherapy was, of course, in mind. However, in the process of working out proper dosage and routes of administration another biological response caught our attention.

We were surprised to note that the presence of a growing melanoma on a mouse partially protected it from the toxicity of PPDA.[4] In order to explore this phenomenon more carefully we worked out the following simplified experimental design: identical groups of mice in respect to genetics, sex, age, and other tangible factors were established, and suitable numbers of them were implanted with melanoma, or other tumor types.

As illustrated in FIGURE 4, the control and tumor-bearing mice were injected, intraperitoneally and alternatively, with identical toxic doses of the test compound on a milligram per kilogram basis, and the time was recorded to the nearest minute. If the challenging compound is distributed uniformly throughout the carcass and the tumor and is otherwise acted upon in the same manner by both groups of mice, the survival time should be the same within experimental limits. These limits have been determined with appropriate controls, and the differences that will be presented have been analyzed and found to be statistically significant.

Following I.P. injection, the animals are observed closely, and the deaths are recorded to the nearest minute. When all mice are dead, or the experiment is otherwise terminated, the survival times are calculated, and the values are ranked and put in graphic array. Without going into statistical details, we have found it useful to employ the median of the group response if we wish to use a single number to express the results. This is actually the cumulative average median (CAM), and it is the most stable single point representing the group response, since it is not influenced by one or two maverick long-time survivors that become difficult to handle intelligently in group averages. Alternatively, the entire cumulative response of the groups can be plotted as shown in FIGURE 5.

This figure shows the protective effect of the pigmented tumor when the mice were challenged with a lethal dose of PPDA. The numbers at the bottom represent the number of mice averaged, and the coordinate

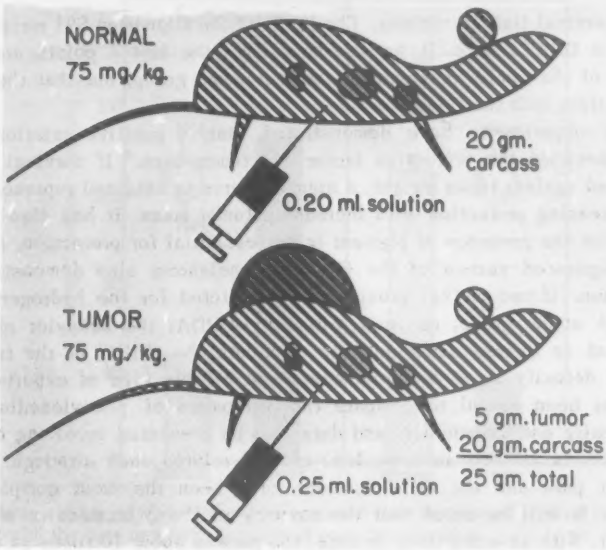

FIGURE 4. Diagrammatic illustration of balanced dose administration to normal and tumor-bearing mice.

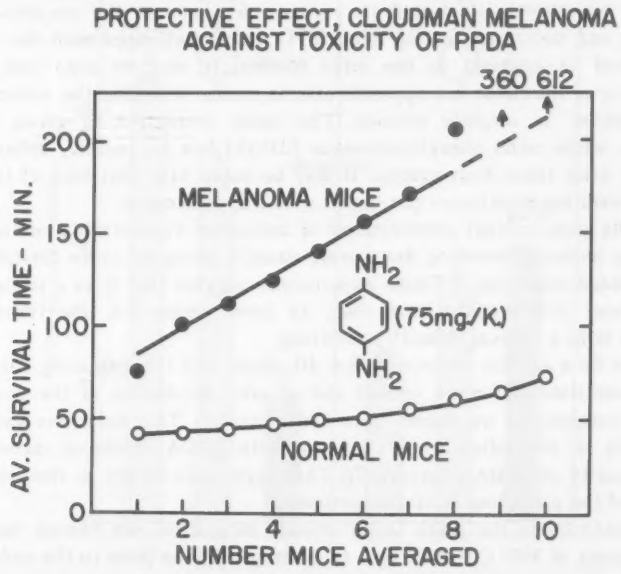

FIGURE 5. Method of plotting cumulative response of normal and experimental groups; CAM (cumulative average median) is the midpoint of each curve.

is the survival time in minutes. The tumor is the Cloudman S91 melanoma grown in DBA/2 mice. It may be noted that the last 2 points are not typical of the family response of the melanoma group, but that they do not interfere with the CAM.

Other experiments have demonstrated that a positive relationship exists between the protection factor and tumor size.[4] If survival time is plotted against tumor weight, a sigmoid curve is obtained representing the increasing protection with increasing tumor mass. It has also been noted that the presence of pigment is not essential for protection, since a nonpigmented variant of the Cloudman melanoma also demonstrates protection. If two methyl groups are substituted for the hydrogens of a PPDA amino group, as in *N,N*-dimethyl-PPDA, the behavior of the compound is altered sufficiently to eliminate the ability of the tumor-host to detoxify it, at least as expressed by this type of experiment.

It has been useful to compare the 3 isomers of phenylenediamine biologically and chemically, and data will be presented involving these 3 compounds as well as some less closely related ones although, thus far, the para and the ortho isomers have been the most completely studied. It will be noted that the toxicity of the 3 isomers is widely different. With an acute dose in mice, the para is about 10 times as toxic as the ortho, while the meta occupies an intermediate position in this regard.

The experiment illustrated in FIGURE 6 compares the response of normal and melanoma-bearing mice (S91) when challenged with the para compound in contrast to the ortho isomer. It may be seen that the presence of the tumor has opposite effects on the host when the molecular arrangement is slightly altered. The usual protection is given with PPDA, while ortho phenylenediamine (OPDA) has its toxicity enhanced by the same tumor-host system. It may be noted also that both of these compounds have antitumor properties with this melanoma.

In the past, casual observations of increased vulnerability of tumor-bearing animals receiving drugs were usually assigned to an amorphous "weakened condition." These experiments suggest that it is a matter of tumor-host biochemistry and that, in some instances, the tumorous animal is in a "strengthened" condition.

If we do a similar experiment but dispense with tumor-bearing animals and substitute melanoma extract and *in vitro* incubation of the para or ortho compounds, we obtain consistent results. The melanoma extract protects or detoxifies when combined with PPDA, while it increases the toxicity of OPDA (FIGURE 7). This appears to be the *in vitro* equivalent of the preceding *in vivo* experiment.

In addition to the fresh tumor extract incubation, we heated half of the extract at 100° C. for 15 min. to destroy enzymes prior to the addition of the phenylenediamines. It is seen that there nevertheless was an appropriate effect with both compounds. The logical interpretation of

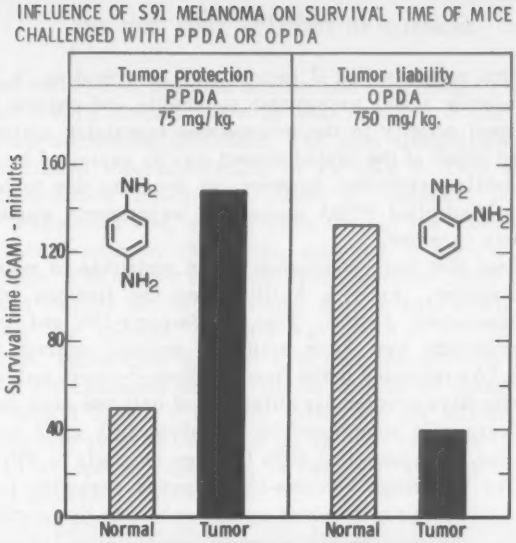

FIGURE 6. Inversion of survival response due to melanoma when comparing toxicity of the para and ortho isomers of phenylenediamine.

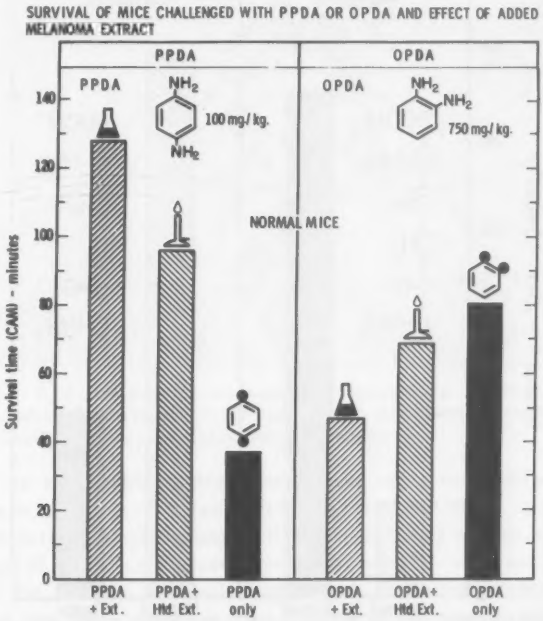

FIGURE 7. Influence of melanoma extract, heated and unheated, on the relative toxicity of para and ortho isomers of phenylenediamine.

these results suggests that 2 factors may be operating, a heat-stable and a heat-labile agent, presumably metabolite and enzyme since there was additional activity in the preparations containing active enzymes. The reduced effect of the heated extract may be explained by destruction of a heat-labile metabolite; however, no decrease due to heating was observed in controlled PPDA manometric experiments employing melanoma extracts or urines.

To indicate that this phenomenon is not restricted to melanomas and phenylenediamines, FIGURE 8 illustrates the findings with another tumor-host-compound system. This is Sarcoma-180 and two closely related compounds that have antitumor activity against this mouse malignancy.[5] As indicated in the figure, 6-diazo-5-oxo-L-norleucine (DON) and azaserine have a molecular difference of only one atom in the carbon chain: an oxygen is substituted for a carbon, with its 2 hydrogens, in the 4 position. It is seen that DON behaves similarly to PPDA in being detoxified by or through Sarcoma-180, whereas azaserine is analagous to OPDA in that this sarcoma-host system enhances its toxicity.

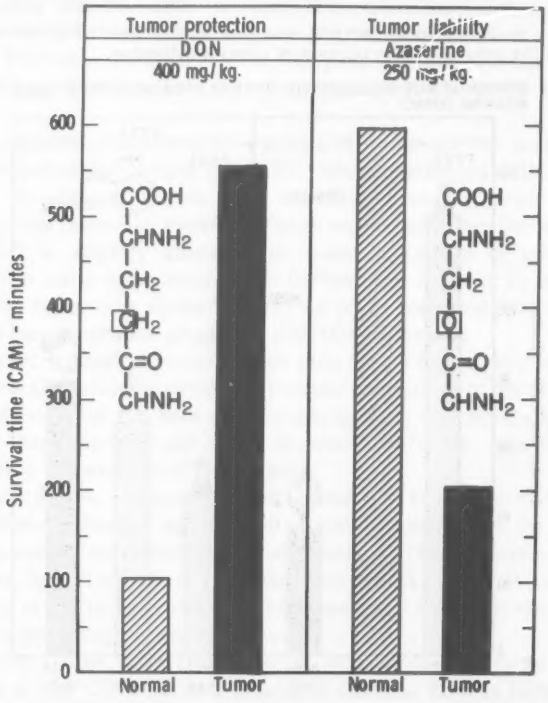

FIGURE 8. Opposite effects of DON and azaserine in respect to the influence of Sarcoma 180 in modifying survival time.

There are a number of possible mechanisms to account for the foregoing phenomena. I shall discuss these in what appears, at the moment, the order of their decreasing likelihood. The most straightforward explanation is illustrated diagrammatically in FIGURE 9. This assumes that the intraperitoneally injected compound is absorbed, goes into the peripheral circulation and, as it passes through the vascularity of the tumor, is acted upon by the unique enzymes or special metabolites localized there. The altered compound then expresses itself by increased or decreased toxicity and is thus either more or less lethal to the vital areas of the host carcass. Although FIGURE 9 is labeled in terms of detoxification, either case applies. It is also theoretically possible, of course, that the compound may be altered without its being detected by this experimental design if toxicity values are not altered. By further analogy, one may think of the tumor as an extra transplantable organ in reference to its special capacities to engage in the biochemical transformation of appropriate compounds.

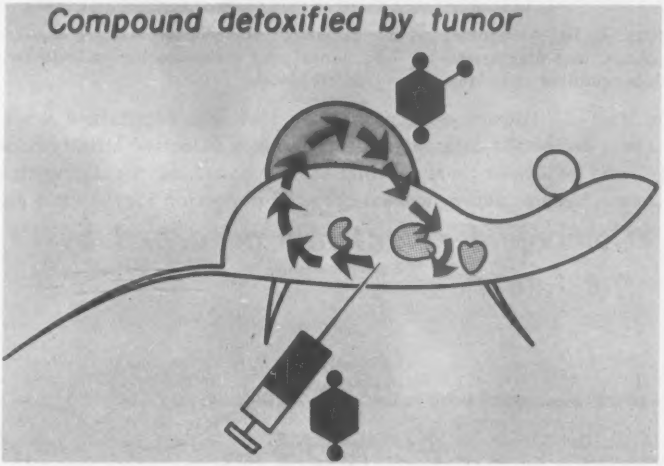

FIGURE 9. A hypothetical mechanism to account for toxicity changes in compounds that distinguish between normal and tumor-bearing animals through tumor metabolites or enzymes associated with tumor.

Since we have evidence that some of the tumor metabolites, as well as enzymes, spill out of the cells into the peripheral blood, it is possible for the reaction to take place in the host vascular system without the necessity of tumor cell penetration (FIGURE 10). However, since we obtained the reaction with relatively blood-free tumor extract, it would seem that the tumor cells are also capable of the transformations. The release of large quantities of certain enzymes into the blood by or because of the tumor will be illustrated later.

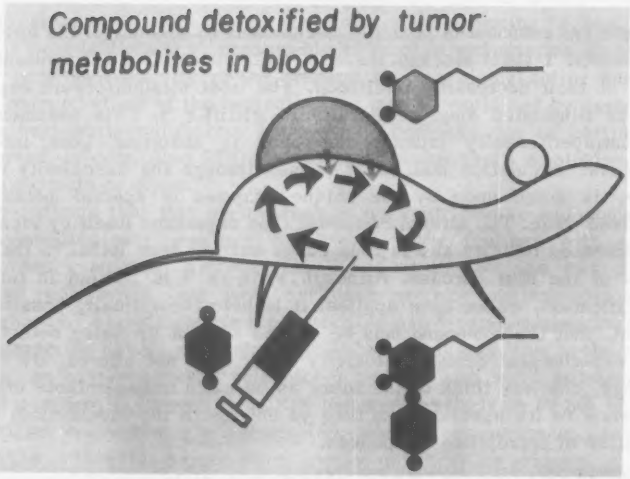

FIGURE 10. A hypothetical mechanism to account for toxicity changes in compounds that distinguish between normal and tumor-bearing animals through tumor metabolites or enzymes in peripheral blood.

FIGURE 11 illustrates a possibility that was entertained when we had tumor protection data only. If there were a selective affinity between PPDA and melanoma tissue so that the compound, in circulating through the tumor, became bound, it would provide protection for the vital organs

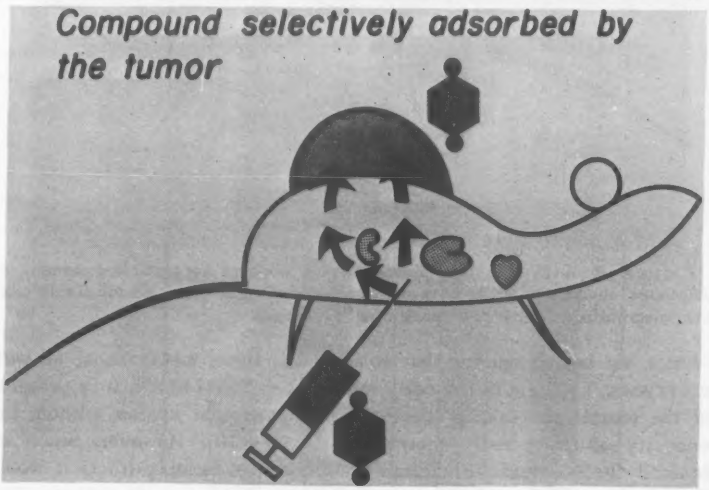

FIGURE 11. A hypothetical mechanism intended to account for toxicity changes in compounds that distinguish between normal and tumor-bearing animals through physical inactivation by binding in tumor tissue.

of the host. However, since this explanation does not apply in the cases where we obtain increased toxicity, its validity would appear to be only partial and in any case limited.

Alterations in liver catalase[10] and recent unpublished evidence of other enzyme alterations in the liver as a consequence of an animal being host to a tumor raise legitimate questions concerning the possibility that the tumor plays an indirect role in these phenomena involving altered toxicity. FIGURE 12 illustrates such hypothetical possibilities. For example, if the liver were deprived of certain enzymes or was fortified with more or different enzymes as a result of the presence of a malignancy, it could be reflected in these experiments. Again, however, the direct effect of tumor extract diminishes the probability that this mechanism is involved. Liver extract from tumor-bearing mice was tested directly in a preliminary experiment without evidence of altering the toxicity of OPDA.

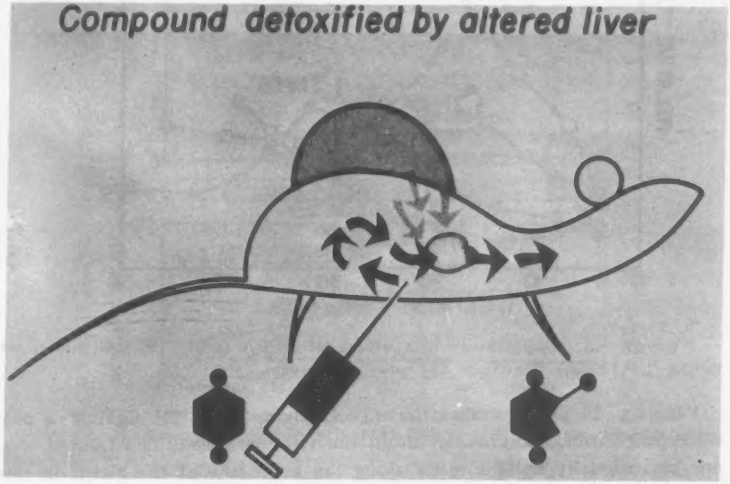

FIGURE 12. A hypothetical mechanism to account for toxicity changes in compounds that distinguish between normal and tumor-bearing animals through enzymic alterations in liver or other organs, induced by tumor.

Space does not permit a complete discussion of the tumor chemotherapy done with the phenylenediamines.[3,6,7] However, to indicate a possible relationship between the reactions we have presented involving tumor-cell components and these compounds as chemotherapeutic agents, I include examples of the antitumor activities of the three isomers. FIGURE 13 illustrates inhibition of the Cloudman S91 mouse melanoma by ortho, meta, and para phenylenediamine. The para and meta compounds (PPDA and MPDA) were given orally in the drinking water at concentrations of 150 mg./100 ml. distilled water, while the ortho compound (OPDA) was

administered intraperitoneally at 200 mg./kg. The tumors were all well established at the time treatment started about 20 days postimplantation.

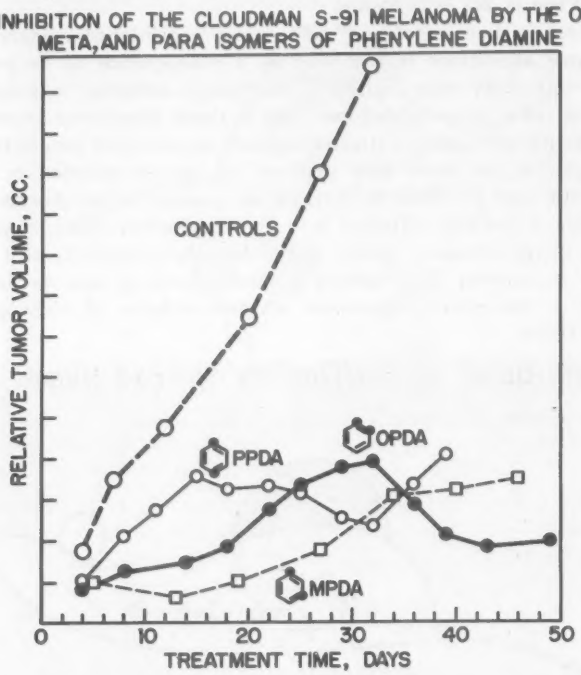

FIGURE 13. Chemotherapeutic effect of PPDA (oral), MPDA (oral), and OPDA (I.P.) on the Cloudman S91 mouse melanoma.

FIGURE 14 demonstrates the effectiveness of OPDA against a non-melanoma tumor, the classic, solid Ehrlich carcinoma. The treated mice are indicated by solid circles along the base line of the chart. In this experiment the OPDA-treated tumors grew slightly and then regressed. The tumors did not reappear following discontinuation of the treatment. There was a survival of about 70 per cent of the treated animals, and these survivors remained tumor-free during several months of observation. No untreated controls survived.

At present we do not have enough observations to establish a positive relationship between chemotherapeutic effectiveness and the tumor protection-liability phenomena. However, with the limited existing data, the successful therapeutic compounds have had either tumor-protection or tumor-liability effects. This may be coincidental.

In collaboration with Felix Wróblewski and Chester Stock, I have been studying the correlation between plasma lactic dehydrogenase activity

(LDH) and tumor growth in experimental animals.[8] The details of these studies will be reported elsewhere,[9] but I mention one aspect of the work in order to add a specific enzyme as another parameter in the tumor-host-molecule relationship that has been described here.

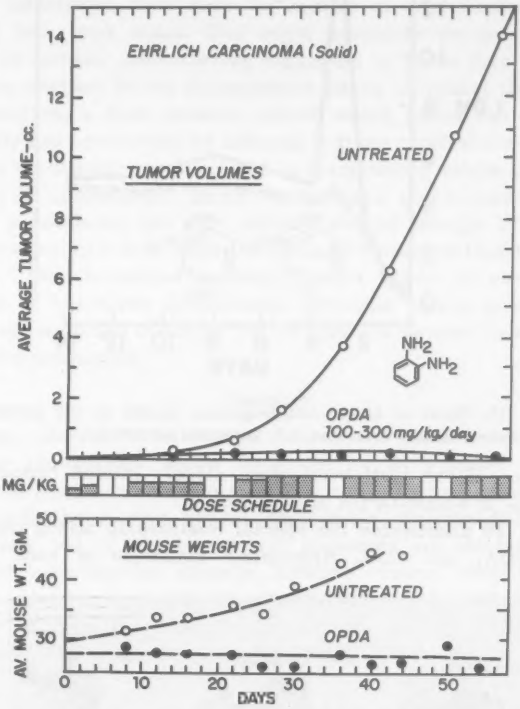

FIGURE 14. Chemotherapeutic effect of OPDA (I.P.) on the Ehrlich solid carcinoma.

We have established by repeated experiments with transplantable tumors that good correlation exists between increasing LDH in the blood plasma and growth of the tumor. If the tumor is inhibited by OPDA or other antitumor compounds, the level of the enzyme in the blood is modified accordingly. However, a phenomenon of special interest in respect to this enzyme and OPDA is observed when the compound is injected I.P. in an animal during the early stages of tumor growth. In contrast to normal animals injected with OPDA, those injected with a tumor or a tumor virus give a burst of LDH in their blood plasma, as though excessive quantities of the enzyme were being eluted or elicited from some organ or tissue of the host. This is illustrated for the Friend leukemia virus in FIGURE 15. The compound specificity is indicated by

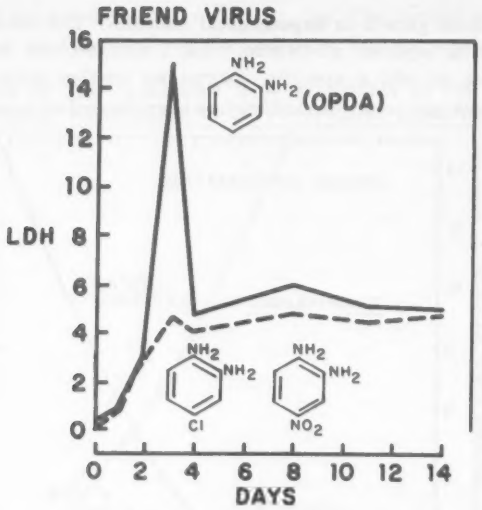

FIGURE 15. Burst of lactic dehydrogenase (LDH) in the peripheral blood of leukemia-infected mice following I.P. injection of OPDA.

the absence of the LDH burst when closely related compounds such as 4-chloro- or 4-nitro-OPDA are injected.

FIGURE 16 summarizes the special relationship among the host, the tumor, OPDA, and LDH. Appropriate extension of such integrating

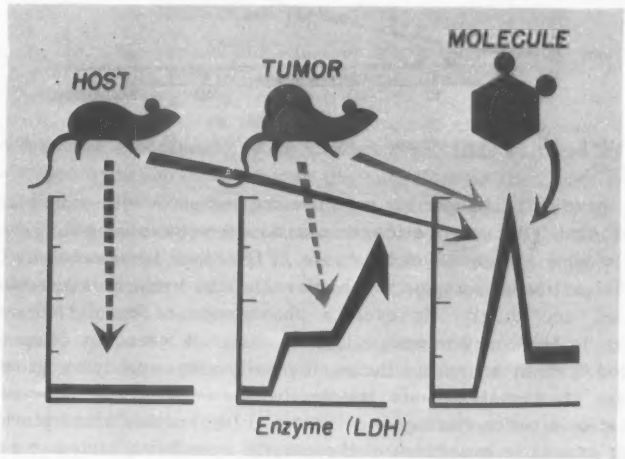

FIGURE 16. Diagrammatic illustration of some special relationships among host, tumor, enzyme, and a specific molecule.

clues should lead to a more useful understanding of some of the intricacies involved in tumor-host differences and relationships. If the intracellular contents of malignant tissues include exploitable components that permit disruptive intracellular reactions to occur when the proper agent is introduced, then hope for control of this disease could be converted into work hours. One might speculate on the avenues of approach to rational chemotherapy suggested by these data and inquire whether, in contrast to the disingenuous attack on cancer through metabolic analogues, a more incisive assault would poison the cancer cell specifically and irreversibly by inducing a direct combination between an exogenous therapeutic compound and an accumulated unique intracellular metabolite or, alternatively, permit the malignant cell to poison itself by providing a substance that the peculiar enzyme linkage of the cancer cell will convert to a more toxic intracellular substance than the injected compound. The theoretical possibility also exists of combining the usefulness of analogues in producing metabolic blocks to generate an enhanced accumulation of unique intracellular substances for exploitation by the above mechanism.

Summary

Following chemical challenge of normal and tumor-bearing mice by appropriate biologically active molecules, it is possible to observe subtle tumor-host, tumor-molecule, and tumor-molecule-enzyme relationships at both the *in vivo* and *in vitro* levels. Striking differences in survival time and in certain enzymic responses are obtained between normal and tumor-bearing animals, between various tumor types, and between administered compounds where there may be an alteration of only one atom or merely an isomeric difference.

Tumor inhibitory properties have also been observed in the compounds tested.

Acknowledgments

I am indebted to Edith Huerto, David Bardell, and Frank Lilly for valuable contributions to these studies. Generous assistance was also provided through the SKI Volunteer Department by Winifred L. Powers, Marie Bunker, Eleanor Hevesi, Claude Arpels, Alan F. Arnold, Henry Abrahams, and Charles Forman. The work involving lactic dehydrogenase (LDH) was done in collaboration with Felix Wróblewski and C. Chester Stock.

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DIVISION OF MYCOLOGY

MYCOPARASITISM OF *GONATOBOTRYUM FUSCUM* AND *PIPTOCEPHALIS XENOPHILA**

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Two biotrophic fungi, *Gonatobotryum fuscum* Sacc.,† parasitic on species of *Ceratocystis*, and *Piptocephalis xenophila* Dobbs and English, parasitic on species of *Penicillium*, were used to study the basic principles of parasitism.

These two fungi parasitic on other fungi (mycoparasites) were chosen for this study because they are unrelated taxonomically and because they showed interesting physiological similarities in preliminary studies. A physiological study of mycoparasitism of *G. fuscum* has not been reported prior to this study. Preliminary studies suggested that the responses of this fungus to changes in nutrition were different from those of other mycoparasites studied.[1-4]

P. xenophila is the only species of this genus that parasitizes fungi in other orders as well as those belonging to the Mucorales.[5] The hosts are penetrated by haustoria. Investigations on the physiology of parasitism using *P. virginiana* have been reported,[3,4] and were useful in planning additional studies on *P. xenophila*.

These and other fungi lend themselves very well to basic studies on parasitism since they can be grown easily in abundance in a small space, since they can be observed under many exacting conditions, and since their responses to changes in nutrition and environment can be measured accurately. They also may be observed microscopically while still living.

Barnett and Lilly[2] pointed out that generally there are four types of mycoparasites recognized, based on the mode of parasitism:

(1) Those that penetrate the host and form haustoria, as in species of *Piptocephalis*.[4-6]

(2) Certain fungi such as *Chaetocladium* sp. and *Parasitella simplex* that dissolve a hole in the host hyphae after contact is made. The host

* This paper, illustrated with slides, was presented at a meeting of the Division on February 26, 1960. It is based in part on the author's doctoral dissertation, West Virginia University, Morgantown, W. Va., 1959, and is published with the approval of the West Virginia Agricultural Experiment Station, Morgantown, W. Va. as Scientific Paper No. 623. This work was supported in part by a grant from the National Science Foundation, Washington, D. C.

† Species verified by Stanley J. Hughes, Senior Mycologist, Department of Agriculture, Ottawa, Ont., Canada.

nuclei pass into a specialized basal cell of the parasite, which continues to grow.[7]

(3) Those that twine around their host and finally penetrate after death of the host. Butler[8] describes this action by *Rhizoctonia solani* on certain species of the Mucorales.

(4) Those fungi that produce antibiotics and arrest the growth of other fungi growing near them. This type of parasitism has received very little attention, and it is suggested by Weindling[9] that *Gliocladium* sp. destroys other fungi in this manner. The possible parasitic nature of *G. roseum* on several other fungi is presented by Tubaki[10] and Shigo.[11] The latter pointed out that *G. roseum* destroyed all fungi that were paired with it in culture.

A fifth type of mycoparasite may be represented by *Calcarisporium parasiticum*. The short branches produced near the host by this fungus and the small buffer cells produced at the point of host-parasite contact are unique in the fungi.[1,2]

The mode of parasitism and host range of *P. xenophila* have been reported.[5] It was necessary to conduct similar basic studies on *G. fuscum* before further work could be planned.

The isolate of *G. fuscum* used was obtained from an oak-wilt tree in West Virginia in 1956. The parasite was always in close association with a species of *Graphium*. Fourteen oak-wilt trees in the same area yielded the two fungi.

Spores of *G. fuscum* were obtained free from spores of the host by the following technique. *Ceratocystis ulmi* was used as the host. The parasite grew beyond the edge of this host, and inoculum could be collected easily.

Quantities of spores of the parasites were often needed for the experiments. The scraping of the young host-plus-parasite cultures with a flat needle stimulated growth and sporulation of both mycoparasites.

Gonatobotryum fuscum grew parasitically when the hosts were grown on a variety of media, but it failed to grow saprophytically free from the host (in axenic culture) on eighty-five different media. Many media contained autoclaved and sterile-filtered extracts and host filtrates from several hosts. Some traces of axenic growth (not typical growth) were obtained with media containing host filtrates, small amounts of yeast extract, and microelements.

The mode of parasitism of *G. fuscum* is similar to *Calcarisporium parasiticum*. Small abundant absorptive hyphae of the parasite attach themselves to the young host hyphae without penetrating the walls, and a balanced type of host-parasitic relationship results. It is conjectured that the center of certain enzyme activities is in these absorptive hyphae. A similar hypothesis concerning *C. parasiticum* has been proposed.[2]

The possibility exists that these absorptive hyphae may function physiologically as do haustoria, although they do not penetrate the

host. The clasping nature of these branches indicates a very close union and, since most fusions are with young hyphae, the cell walls of host and parasite are immature; therefore, thinner walls separate protoplasts of the two fungi at these points of contact.

An unusual and unexpected reversal of parasitism appeared to occur in cultures two to four weeks old. Old aerial mycelium and conidiophores of *G. fuscum* were commonly overgrown and attacked by *Graphium* sp., suggesting a change in the nutritional relationship after the available nutrients in the medium were exhausted. The parasite inhibited synnemata growth of *Graphium* sp., and the reappearance of synnemata after the reversal of parasitism suggested that pyridoxine (essential for normal growth and sporulation of *Graphium* sp.) may have been obtained by *Graphium* sp. from *G. fuscum* hyphae.

The formation of thickened areas on the aerial hyphae of *G. fuscum* when parasitism is reversed suggests a protective mechanism against penetration by *Graphium* hyphae. Godfrey[12] offers a similar explanation for the thickened areas in the chlamydospores of *Endogone* spp. A similar reversal of parasitism has been reported with ectotrophic mycorrhiza of trees and endotrophic ones of the orchids. Another example has been reported by Hawker *et al.*[13] in which *Pythium ultimum*, a parasite on *Allium ursinum*, is later arrested and sometimes destroyed by the host.

All fourteen species of *Ceratocystis* tested were hosts for *G. fuscum*, but the parasite's action was not the same on all these hosts. Some hosts supported better parasite growth than the others. Investigators have reported different degrees of parasitism on different hosts with *C. parasiticum*[2] and *P. virginiana*.[3,4] Host physiology is an important factor in the host-parasite complex. Most of the species of *Ceratocystis* have one or more partial or total deficiencies of one or more vitamins. A deficiency may greatly influence the degree of parasitism.

Since *G. fuscum* also parasitizes *Graphium* sp. and *Leptographium* sp., the possibility that they have perfect stages belonging to the genus *Ceratocystis* is suggested. A means of separating two genera, both having a *Graphium* imperfect stage, then might be possible by observing whether *G. fuscum* will parasitize the questionable species. Two species of *Petriella* having a *Graphium* imperfect stage were tested. *G. fuscum* attacked neither of them.

Light was necessary for normal production of synnemata by *Graphium* sp., but constant light inhibited growth of the parasite. The establishment of *G. fuscum* on *Graphium* sp., usually after seven days, resulted in the inhibition of synnemata production. On the other hand, total darkness inhibited synnemata production, but stimulated growth of the parasite.

Light has been shown to affect the degree of parasitism of various mycoparasites. Butler[8] pointed out that *Rhizopus* sp., *Mucor recurvus*, *Pythium debaryanum*, and *P. butleri* were more susceptible to attack by

Rhizoctonia solani in total darkness than in constant light. Parasitism of *Graphium* sp. by *G. fuscum* was stimulated in darkness only between 10° C. and 28° C. The presence of light did not influence parasitism at temperatures above 29° C. Optimum conditions for growth of *G. fuscum* on several hosts were 20° C. and total darkness. Some enzyme systems are more active at specific temperatures than at others.[14] Light can also activate certain enzyme systems.[15] No doubt, the factors affecting the enzyme systems of the host in turn are directly responsible for the action of the parasite.[16]

All experiments with *P. xenophila* were with the original host, *Penicillium frequentans*, received from C. R. Berry, Asheville, N. C. *P. virginiana* was cultured on *Mortierella pusilla*. A detailed description of the latter mycoparasite has been reported.[3,4,6]

The carbon-nitrogen ratio of the medium affected the degree of parasitism. A 5:5 glucose-yeast extract agar medium (5 gm. each of glucose and yeast extract per liter) was excellent for growth of *P. xenophila* on *P. frequentans*, while parasitism was poor on a similar (5:1) medium. Similar results have been reported for *P. virginiana*.[3,4] Mycelial dry weights of host-plus-parasite cultures were less than those cultures of host alone on the same media. On the contrary, *G. fuscum* on *Graphium* sp. and *C. ulmi* grew well on the 5:1 medium and poorly on the 5:5 medium. On the host *Leptographium* sp., *G. fuscum* responded similarly to *P. xenophila* on the two media. These results indicate that host physiology to a great extent determines the degree of parasitism.

An effect of the concentration of microelements on parasitism was discovered when as little as 0.1 mg. of manganese per liter (as manganese sulfate) was added to the above 5:5 medium, and *G. fuscum* grew rapidly on *Graphium* sp. and *C. ulmi*. It is difficult to assume that the high concentrations of manganese added were required by the hosts, since the hosts were not altered macroscopically in these media. It is believed that the metal acted in some way that inhibited or increased production of certain materials necessary for parasitic growth of *G. fuscum*. Since *G. fuscum* grew on *Graphium* sp. and *C. ulmi* on the 5:1 medium but not on the 5:5 medium, these results suggest that the addition of more yeast extract increases certain materials that indirectly inhibit parasitism. Investigations on enzyme action by Swartz *et al.*[17] and Sizer[18] help to support the above views.

The addition of at least 4 ml./l. of a stock solution of microelements* to a 5:5 glucose-yeast extract liquid medium was necessary for good growth of *P. xenophila* on *P. frequentans* and *P. virginiana* on *M. pusilla*. On agar, the same medium without the added microelements supported excellent parasite growth. All indications were that agar contained certain materials necessary for parasitic growth. The excellent growth

*Two ml. of the microelement stock solution contained 0.2 mg. each of Zn and Fe, 0.01 mg. of Mn, and 20 mg. of Ca.

of the parasite in liquid media plus the microelements suggested that the metals in the agar were the necessary substances. The addition of several metals was more effective than manganese alone with *P. xenophila* and *P. virginiana*. These results indicate a major role of microelements in the host-parasite complex.

The possibility that microelements have a pronounced effect on the degree of parasitism was suggested by Sadasivan,[19] who demonstrated that manganese stimulated bacterial growth and sporulation of *Fusarium vasinfectum* in the soil. Sulochana[20] also observed that zinc was effective in reducing the wilt incidence on cotton incited by *F. vasinfectum*, whereas manganese aggravated the incidence of wilt.

A series of experiments was made using liquid media in which the source of nitrogen was varied. The media contained per liter 5 gm. of glucose, the usual amount of salts and microelements, and either potassium nitrate, asparagine, or ammonium nitrate in amounts that supplied the same amount of nitrogen as 5 gm. of yeast extract. The 3 nitrogen sources each supported good parasitism of *P. xenophila*, provided a relatively large amount of microelements was added to the control media. The parasite usually grew on hosts in the control media, not amended with the extra microelements, but never as well as in the microelement-amended media. The parasite always appeared several days earlier in the microelement-amended media. These results indicate that time is also an important factor to be considered. It is possible that the addition of the microelements increased the rate of certain reactions and, in doing so, brought about conditions optimum for parasitism at an earlier period. *P. frequentans* sporulated sooner in the microelement-amended media, but sporulation of the host was arrested in areas where the parasite was sporulating.

Several amino acids, on an equal nitrogen basis, were utilized differently by the hosts, and there were also varied degrees of parasitism in these nitrogen sources. Glutamic acid was of particular interest. A 5:5 glucose-glutamic acid basal agar medium (5 gm. of glucose and an amount of glutamic acid necessary to yield the same amount of nitrogen as in 5 gm. of yeast extract) supported good parasitism of *P. virginiana* on *M. pusilla*, but not of *P. xenophila* on *P. frequentans*. On a similar 5:1 medium the results were completely reversed. It should be pointed out that the nitrogen source and carbon source are important factors affecting host physiology and consequently the degree of parasitism.

The importance of the carbon-nitrogen ratio has been stressed by other investigators.[2-4] It has been pointed out that different ratios of the same carbon and nitrogen sources can alter the degree of parasitism. Micro-Kjeldahl analyses of the nitrogen content of the trichloroacetic acid extract of the mycelium of hosts growing in a poor medium were compared with those of mycelium growing on a good medium for para-

sitism. The results indicated that the mycelium from a medium supporting good parasitism contained a higher percentage of trichloroacetic acid-nitrogen, on a dry mycelial weight basis, than the mycelium grown in a medium not supporting parasitism.

The amount of nitrogen available to an organism and the level of the amino acid pool are postulated to affect enzyme synthesis.[16,21] The possibility of an internal dynamic carbon-nitrogen ratio could be considered, and the ability of a parasite to infect would depend on the proper balance of that ratio.

The respiratory rise in fungus-infected tissues of phanerogams has been pointed out by several investigators.[22-25] No report of similar studies on fungi parasitized by other fungi has been found.

To accomplish such respiration studies, a modified method and apparatus described by Thimann *et al.*[26] were used. Host or host-plus-parasite spores, or both, of several fungi were spread evenly over the longitudinal surface of 2 ml. of agar medium in a 5-ml. screw-cap vial. Under aseptic conditions each vial was placed into a 30-ml. glass tube containing filter paper saturated with a 20 per cent potassium hydroxide solution. A 5-ml. serologic pipette was placed in a rubber stopper that sealed the large tube. This apparatus was then placed in a 28° C. constant-temperature water bath. The amount of oxygen utilized was indicated in the pipettes by water displacement. At least 6 replicates of each variable were used in each experiment. The average amount of water drawn into the control pipettes lacking inoculum was subtracted from the experimental systems in order to obtain the correct amount of oxygen utilized. All results were recorded on a relative basis, and the amount of oxygen used under standard conditions was not calculated.

It was concluded from the respiration studies that a fungus infected or parasitized by another fungus respires at a rate higher than normal. Realizing that this is true with the fungi is of great benefit, since large-scale experiments can be conducted with relative ease and the results possibly applied to fungus-infected higher plants.

Host nutrition and environment greatly affected the degree of parasitism. The mycoparasites responded differently to the addition of microelements to the medium. The addition of manganese to the medium stimulated growth of *G. fuscum* on its hosts. The carbon-nitrogen ratio of the medium, as well as light and temperature, affected the degree of parasitism.

The use of mycoparasites to study the basic principles of parasitism has many advantages; additional necessary information on parasitism can be obtained by more intensive investigations on the many different types of mycoparasites.

Acknowledgment

The assistance of H. L. Barnett and V. G. Lilly during the course of the investigation is gratefully acknowledged.

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CONFERENCES HELD

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

FOURTH TISSUE HOMOTRANSPLANTATION CONFERENCE

Thursday, February 4
Friday, February 5 and
Saturday, February 6, 1960

Conference Co-Chairmen

John Marquis Converse and Blair O. Rogers
New York University College of Medicine
New York, N. Y.

THURSDAY, FEBRUARY 4, 1960

IMMUNOGENETICS AND PHYLOGENETICS

Session Chairman: E. J. Eichwald
Montana Deaconess Hospital
Great Falls, Mont.

9:00 A.M. –
Greetings from the Academy – Charles Noback, Vice-Chairman, Section of Biological and Medical Sciences, The New York Academy of Sciences, New York, N. Y.

Introductory Remarks – John Marquis Converse, New York University College of Medicine, New York, N. Y.

"Dosage and Additive Effects of Histocompatibility Genes in the Teleost *Xiphophorus maculatus*" – Klaus D. Kallman, New York Zoological Society Genetics Laboratory, New York, N. Y.

"Sexual Dimorphism in the Histocompatibility Reactions of Amphibia to Skin Homografts and a Tentative Explanation of Their Mechanism" – Donald Pizzarello and Alexander Wolsky, Biological Laboratory, Fordham University, New York, N. Y.

"Chronic Skin Homograft Rejection in the Syrian Hamster" – W. H. Hildemann and R. L. Walford, University of California School of Medicine, Los Angeles, Calif.

"Age Factor and Induction to Immunological Tolerance to Male Skin Isografts in Female Mice Subsequent to the Neonatal Period" – T. Mariani, C. Martinez, J. M. Smith, and R. A. Good, University of Minnesota Medical School, Minneapolis, Minn.

"A Strain-Specific Influence of Parity on Resistance to Homografts" – E. J. Breyere and M. K. Barrett, National Cancer Institute, Public Health Service, Bethesda, Md.

"The Anemia of Parabiotic Intoxication" – E. J. Eichwald, J. P. Pfaff, Jr., and E. C. Lustgraaf, Montana Deaconess and Columbus Hospitals, Great Falls, Mont.

VARIATIONS IN THE RESPONSE TO TISSUE TRANSPLANTS

Session Chairman: Merrill W. Chase
The Rockefeller Institute
New York, N. Y.

2:00 P.M. –

"Enhancing Effect upon Homografts of Skin by Surgical Ablation of Regional Lymph Nodes" – Richard B. Stark and Theodore Dwyer, St. Luke's Hospital, New York, N. Y.

"Suppression of Transferred Lymph Node Cells in Neonatal Rabbits by the Injection of Leukocytes" – T. N. Harris and Susanna Harris, The Children's Hospital of Philadelphia, Philadelphia, Pa.

"Concerning Some Peculiarities of the Behavior of Cutaneous Homotransplants Depending on Their Size" – E. A. Zotikov, Institute of Experimental Biology, U.S.S.R. Academy of Medical Science, Moscow, Union of Soviet Socialist Republics.

"Some Studies on the Antigenicity of Cartilage" – Erle E. Peacock, Jr., Paul Weeks, and Jerry Petty, School of Medicine, University of North Carolina, and the North Carolina Memorial Hospital, Chapel Hill, N. C.

"An Investigation of Hodgkin's Disease with Respect to the Problem of Homotransplantation" – William D. Kelly, Robert A. Good, Donald L. Lamb, and Richard L. Varco, Veteran's Administration Hospital and the University of Minnesota, Minneapolis, Minn.

"The Specificity of the White-Graft Reaction in Man" – F. T. Rapaport, L. Thomas, John Marquis Converse, and H. S. Lawrence, New York University College of Medicine, New York, N. Y.

FRIDAY, FEBRUARY 5, 1960

ANTIGEN-ANTIBODY MANIFESTATIONS IN TISSUE TRANSPLANTATION

Session Chairman: William S. Tillett
New York University College of Medicine
New York, N. Y.

9:00 A.M. –

"The Transfer of Sensitivity to Skin Homografts in Man with Leukocyte

Extracts" – H. S. Lawrence, F. T. Rapaport, John Marquis Converse, and W. S. Tillett, New York University College of Medicine, New York, N. Y.

"Passive Transfer of Allergic Reactions to Tuberculin with Plasma Protein Fractions from Hypersensitive Guinea Pigs" – Helene C. Rauch and Cutting B. Favour, Palo Alto Medical Research Foundation, Palo Alto, Calif.

"Humoral Aspects of the Immune Response to Homografts" – Chandler B. Stetson and Emmerik Jensen, New York University College of Medicine, New York, N. Y.

"Demonstration of a Cytotoxic Antibody *in Vitro* Following the Rejection of Skin Grafts by the Rabbit" – John P. Merrill and E. Freidman, Peter Bent Brigham Hospital, Boston, Mass.

"Limitations of the Cytotoxic Effects of Isoantibody" – D. B. Amos, Roswell Park Memorial Institute, Buffalo, N. Y.

"Preparation of a Transplantation Antigen from Epidermal Cells" – F. Albert and G. Lejeune-Ledant, Service de Pathologie et de Thérapeutique Chirurgicales, Université de Liège, Liège, Belgium.

"Effects of Antibody and Complement of Permeability Control in Mammalian Cells" – Howard Green and B. D. Goldberg, New York University College of Medicine, New York, N. Y.

GRAFT VERSUS HOST REACTIONS AND IMMUNOLOGICAL TOLERANCE

Session Chairman: Hilary Koprowski
The Wistar Institute
Philadelphia, Pa.

2:00 P.M. –

"Some Experiments on Acquired Immunological Tolerance in Goats" – B. C. G. Carter and B. Cinader, University of Toronto, Toronto, Ont., Canada.

"Immune Hemolysis: A Feature of 'Secondary Disease' and 'Runt Disease' in the Rabbit" – K. A. Porter, St. Mary's Hospital Medical School, London, England.

"Acquired Tolerance to Homologous Skin Grafts in the Human Infant at Birth" – R. Fowler, Jr., W. K. Schubert, and C. D. West, The Children's Hospital Research Foundation, Cincinnati, Ohio.

"The Effects of Nucleic Acids on Homograft Tolerance" – Franklin L. Ashley, Earl G. McNall, N. R. Dutt, Eugene N. Garcia, and Robert F. Sloan, University of California Medical Center at Los Angeles, Los Angeles, Calif.

"Runt Disease" – G. Siskind and L. Thomas, New York University College of Medicine, New York, N. Y.

"Studies on the Induction of Tolerance of Homologous Tissues and on 'Runt' Disease in Rats" – R. E. Billingham, Jean Brown, V. Defendi, W. K. Silvers, and D. Steinmuller, The Wistar Institute, Philadelphia, Pa.

SATURDAY, FEBRUARY 6, 1960

ORGAN TRANSPLANTATION

Session Chairman: Joseph E. Murray
Peter Bent Brigham Hospital
Boston, Mass.

9:00 A.M. –

"Transplantation of Homologous Parathyroid Gland in the Treatment of Chronic Tetany" – Julian A. Sterling, Albert Einstein Medical Center, Philadelphia, Pa.

"Endocrine Tissue Homotransplantation" – John R. Brooks, Somers H. Sturgis, and George J. Hill, Peter Bent Brigham Hospital, Boston, Mass.

"Transfusion Transplantation of Cadaveric Blood" (On the Thirtieth Anniversary of the Clinical Use of this Method) – Mikhail M. Tarasov, Sklifosovsky Institute, Moscow, Union of Soviet Socialist Republics.

"The Relationship of Serum Globulin Fractions to Renal Transplant Rejection in the Dog, Studied by Fluorescent Antibody and Electrophoretic Techniques" – Clark D. West, Robert Fowler, Jr., and Paul Nathan, the Children's Hospital Research Foundation and the University of Cincinnati College of Medicine, and the May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio.

"Recent Experiments with Homotransplantation of Refrigerated and Non-refrigerated Kidneys in Dogs" – A. G. Lapchinsky, Institute of Experimental Surgical Apparatus and Instruments, Moscow, Union of Soviet Socialist Republics.

"Total Body Irradiation in Man: Tissue Patterns Observed in Attempts to Increase Receptivity of Renal Homografts" – Joseph E. Murray, John P. Merrill, J. D. Dealy, and G. J. Dammin, Peter Bent Brigham Hospital, Boston, Mass.

Robert L. Kroc
Chairman

Charles Noback
Vice-Chairman

SECTION OF CHEMICAL SCIENCES

AMINO ACIDS, PEPTIDES, AND PROTEINS

Thursday, February 25 and
Friday, February 26, 1960

Conference Chairman: Karl Folkers
Merck & Co., Inc., Rahway, N.J.

THURSDAY, FEBRUARY 25, 1960

INTRODUCTION

9:00 A.M. -
Greetings from the Academy - Frederick R. Eirich, Chairman, Section of Chemical Sciences, The New York Academy of Sciences, New York, N.Y.; Brooklyn Polytechnic Institute, New York, N.Y.

Introductory Remarks - Karl Folkers, Conference Chairman, Merck & Co., Inc., Rahway, N.J.

Keynote Address - "Experiences in the Polypeptide Field: Insulin to Oxytocin" - Vincent du Vigneaud, Cornell University Medical School, New York, N.Y.

ISOLATION AND CHARACTERIZATION

Session Chairman: Paul H. Bell
Lederle Laboratories, Pearl River, N.Y.

"Ion-Exchange Chromatography of Insulin and Other Proteins in Buffers Containing Urea" - R. D. Cole, University of California, Berkeley, Calif.

"Homogeneity Studies with Insulin and Related Substances" - L. C. Craig, The Rockefeller Institute, New York, N.Y.

"The Rapid Determination of Molecular Weights of Peptides and Proteins" - David Yphantis, The Rockefeller Institute, New York, N.Y.

DEGRADATION AND STRUCTURE

Session Chairman: Norman G. Brink
Merck & Co., Inc., Rahway, N.J.

2:00 P.M. -
"Phenylthiohydantoins in Protein Analysis" - Pehr Edman, St. Vincent's School of Medical Research, Melbourne, Australia.

"Structure of Ribonuclease" - C. H. W. Hirs, Brookhaven National Laboratory, Upton, N.Y.

"The Role of the α-Helix in Protein Structure" - Paul Doty, Harvard University, Cambridge, Mass.

"Specific Recombination of the Subunits of Hemoglobin" - Harvey A. Itano, Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

FRIDAY, FEBRUARY 26, 1960

ORGANIC SYNTHESIS OF PEPTIDES

Session Chairman: John C. Sheehan
Massachusetts Institute of Technology, Cambridge, Mass.

9:00 A.M. -

"Synthesis of Peptides" - Miklos Bodanszky, Squibb Institute for Medical Research, New Brunswick, N.J.

"Activated Cyclic Derivatives of Amino Acids in Peptide Synthesis" - John C. Sheehan, Massachusetts Institute of Technology, Cambridge, Mass.

"Conformational Effects in Low Molecular Weight Peptides" - Murray Goodman, Polytechnic Institute of Brooklyn, Brooklyn, N.Y.

"New Approaches to Peptide Synthesis" - George W. Anderson, Lederle Laboratories, Pearl River, N.Y.

"Synthesis of Melanocyte-Stimulating Hormone Analogues" - Klaus Hofmann, University of Pittsburgh, Pittsburgh, Pa.

BIOSYNTHESIS OF PROTEINS

Session Chairman: Fritz Lipmann
The Rockefeller Institute, New York, N.Y.

2:00 P.M. -

"Amino Acid Path in Protein Syntheses" - Paul Zamecnik, Massachusetts General Hospital, Boston, Mass.

Discussant: Daniel Nathans, The Rockefeller Institute, New York, N.Y.

"Protein Synthesis in Cell Nuclei" - Vincent Allfrey, The Rockefeller Institute, New York, N.Y.

Discussant: Severo Ochoa, New York University College of Medicine, New York, N.Y.

"Separation of Amino Acid-Specific s-RNAs" - Robert W. Holley, Cornell University, Ithaca, N.Y.

Discussant: G. David Novelli, Oak Ridge National Laboratory, Oak Ridge, Tenn.

"Synthetic Aspects of Ribosomes in *Escherichia coli*" - Richard B. Roberts, Carnegie Institution, Washington, D.C.

Discussant: Alfred Tissieres, Harvard University, Cambridge, Mass.

Frederick R. Eirich
Chairman

Everett S. Wallis
Vice-Chairman

NEW MEMBERS

Elected February 18, 1960

LIFE MEMBERSHIP

Auerswald, Wilhelm, M.D., Protein Metabolism. Head, Laboratory of Metabolism and Immunological Research, Vienna University Medical School, Vienna, Austria.

Thompson, William T., Jr., M.D., Internal Medicine. Chairman, Department of Medicine, Medical College of Virginia, Richmond, Va.

ACTIVE MEMBERSHIP

Aihara, Kaoru, M.D., Endocrinology. Resident Physician, Pathology, D. C. General Hospital, Washington, D. C.

Aizawa, Miki, M.D., Cancer. Visiting Fellow, Sloan-Kettering Institute, New York, N. Y.

Alexander, Herman D., Ph.D., Biochemistry. Assistant Animal Pathologist, Auburn University, Auburn, Ala.

Alleva, John J., Ph.D., Biology. Senior Biochemist, Smith, Kline & French Laboratories, Philadelphia, Pa.

Anz, Umbert E., M.D., Obstetrics and Gynecology. Private Practice, Los Angeles, Calif.

Arditi, Lucien I., M.D., Internal Medicine. Instructor, Medicine, Cornell University Medical School, New York, N. Y.

Bach-y-Rita, Paul, M.D., Neurophysiology. Post-Doctoral Mental Health Trainee, United States Public Health Service, Los Angeles, Calif.

Barsky, James, Ph.D., Biochemistry. Manager, Research Division, Ethicon Inc., Somerville, N. J.

Battisto, Jack R., Ph.D., Immunology. Assistant Professor, Microbiology and Immunology, Albert Einstein College of Medicine, New York, N. Y.

Bender, Sheldon R., M.D., Cardiology. Associate, Medicine, Hahnemann Medical College & Hospital, Philadelphia, Pa.

Berkowitz, Daniel W., D.C., Chiropractic Research. Private Practice, Belle Harbor, N. Y.

Bernstein, Harry, Ph.D., Cultural Anthropology. Professor, History, Brooklyn College, Brooklyn, N. Y.

Beuzeville, Carlos F., M.D., Biochemistry. Associate Professor, Biochemistry, Facultad De Medicina De Arequipa, Arequipa, Peru.

Bittle, James L., D.V.M., Microbiology. Virologist, Virus Research Laboratories, Pitman-Moore Co., Indianapolis, Ind.

Blanchette, James E., M.D., Psychiatry. Staff Psychiatrist and Chief, Patton State Hospital, Patton, Calif.

Blum, Alvin Seymour, Sc.B., Radioisotopes in Biochemistry and Medicine. Principal Scientist, Coral Gables Veterans Administration Hospital, Coral Gables, Fla.

Bolinger, Robert E., M.D., Metabolism. Associate Professor, Medicine, University of Kansas Medical Center, Kansas City, Kans.

Brown, Earlene, Ph.D., Medicochemical Research. Assistant Professor, Biochemistry, Howard University College of Medicine, Washington, D. C.

Brunaud, Marcal, DVM, Physiology and Pharmacology. Director, des Recherches

Physiologiques des Etablissements Clin-Byla, Paris, France.

Butler, Patrick G., M.D., Dermatology. Private Practice, San Francisco, Calif.

Cahal, Dennis Abraham, M.D., Medical Research. Regional Scientific Director, Squibb Institute for Medical Research, London, England.

Caleel, George T., B.S., Clinical Radioactivity. Assistant Professor, Pharmacology, Chicago College of Osteopathy, Chicago, Ill.

Capriles, Tulio Villalobos, M.D., Hematology. Medical Director, Blood Bank, Banco De Sangre Plaza Hospital Vargas, Caracas, Venezuela.

Carroll, Marcus N., Jr., Ph.D., Neuropharmacology. Neuropharmacologist, A. H. Robins Co., Richmond, Va.

Chichester, C. O., Ph.D., Biochemistry of Pigments. Assistant Professor, University of California, Davis, Calif.

Cox, George E., M.D., Anesthesiology. Anesthesiologist, Private Practice, Chattanooga, Tenn.

Cutting, C. C., M.D., Medical Administration. Executive Director, The Permanente Medical Group, Oakland, Calif.

De Boislambert, Pierre Henri, M.D., Medical Research. Regional Scientific Director, Squibb Institute for Medical Research, Seine, France.

Debus, David A., B.A., Virology. Virologist, Merck Sharp & Dohme, West Point, Pa.

Derrick, John Brian D., Ph.D., Biochemistry. Laboratory Executive Officer, Clinical Investigation Unit, London, Ont., Canada.

Dietz, Nicholas, Jr., Ph.D., Biochemistry. Professor, Biological Chemistry, Creighton University School of Medicine, Omaha, Nebr.

Dixon, Glen J., Ph.D., Biochemistry. Head, Virus Section, Southern Research Institute, Birmingham, Ala.

Dolan, Michael F., M.D., Biochemistry. Graduate Student, Johns Hopkins University, Baltimore, Md.

Dunn, William Lawrie, M.D., Experimental Pathology. First Assistant Resident, Pathology, Massachusetts General Hospital, Boston, Mass.

Dutz-Kohout, Elfriedo, M.D., Pathology. Fellow, Pathology, Francis Delafield Hospital, New York, N. Y.

Eichenberger, Erwin, M.D., Physiology. Head, Pharmacological Department, A. Wander Research Institute, Bern, Switzerland.

Erickson, Roy Vincent, B.S., Parasitology. Instructor, Science, St. John's Preparatory High School, Brooklyn, N. Y.

Estores-Anzaldo, Felicidad, B.S., Pharmacology. Medical Research Center, National Institute of Science and Technology, Manila, Philippines.

Fagan, William Thomas, M.D., Medicine. Assistant Professor, Clinical Urology, University of Vermont, Burlington, Vt.

Farbro, Patrick C., M.S., Psychology. Manager, Professional Personnel Programs, Radio Corporation of America, Camden, N. J.

Feams, Edward Cranshaw, B.S., Analysis of Perfume and Flavor Material. Manager, Analytical Department, van Ameringen-Haebler, Union Beach, N. J.

Fisher, Alexander A., M.D., Dermatology. Associate Professor, Dermatology, University Hospital, Bellevue Medical Center, New York, N. Y.

Garofalo, James Louis, M.D., Clinical Research. Associate Director, Clinical Research, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

Gelbard, Alan, Ph.D., Microbial Physiology. Instructor, Department of Microbiology, State University of New York, Brooklyn, N. Y.

Gildenhorn, Hyman L., M.D., Radiology. Chief, Department of Diagnostic Roentgenology, City of Hope Medical Center, Duarte, Calif.

Gottsch, Lida G., M.D., Epidemiology. Instructor, IPMR, New York University-Bellevue Medical Center, New York, N. Y.

Haag, Fritz M., D.V.M., Physiology. Head, Sterility Research Station, Southwest Foundation for Research and Education, San Antonio, Texas.

Heldt, Thomas J., M.D., Neurology. Senior Consultant, Henry Ford Hospital, Detroit, Mich.

Herion, John C., M.D., Medicine. Instructor, Medicine, University of North Carolina School of Medicine, Chapel Hill, N. C.

Hollis, Robert H., V.M.D., Veterinary Medicine. Manager, Veterinary Department, Abbott Laboratories, North Chicago, Ill.

Horovitz, Zola P., Ph.D., Pharmacology. Research Neuropharmacologist, Squibb Institute for Medical Research, New Brunswick, N. J.

Hwang, Jen, Ph.D., Animal Pathology. Duck Disease Specialist, New York State Veterinary College, Cornell University, New York, N. Y.

Ingenito, Alfonse, B.S., Pharmacology. Teaching Assistant, Rutgers University, New Brunswick, N. J.

Janssen, Paul A. J., Ph.D., Medicinal Chemistry. Director, Research Laboratorium, Beerse, Belgium.

Kertesz, Zoltan I., Ph.D., Plant Biochemistry. Professor, Chemistry, New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y.

Kim, Ki Ho, M.D., Physical Medicine. Assistant to Medical Director, Kessler Institute for Rehabilitation, West Orange, N. J.

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Kuemmerle, Helmut-Paul, M.D., Medicine. University Frauen-Klinik, Tuebingen, Germany.

Kumnick, Lillian S., Ph.D., Autonomic Functions. Research Assistant, Ophthalmology, Lenox Hill Hospital, New York, N. Y.

Lauriat, Charles, M.D., Biological and Medical Sciences. Scientific Director, Institut De Serotherapie, Paris, France.

Lee, John Robert, Jr., M.D., Pediatrics. Instructor, Pediatrics, New York Medical College-Flower Fifth Avenue Hospitals, New York, N. Y.

Lee Y. Chiung Puh, Ph.D., Physiology and Physiological Chemistry. Established Investigator, Minnesota Heart Association, Minneapolis, Minn.

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Marilley, Ralph Joseph, M.D., Internal Medicine. Private Practice, Watertown, N. Y.

Matheson, Alastair T., Ph.D., Structure and Biosynthesis of Proteins and Nucleic Acids. Research Associate, Johns Hopkins University, Baltimore, Md.

Melup, Zula, B.A., Medical Sciences. Interior Designer, New York, N. Y.

Mendoza, Hemán, M.D., Endocrinology. Assistant Professor, Internal Medicine, Universidad Nacional, Colombia, South America.

Morrison, Bayard H., III, M.D., Cancer Chemotherapy. National Service Center, National Institutes of Health, Bethesda, Md.

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Query, Richard Zimri, Jr., M.D., Internal Medicine. Private Practice, Charlotte, N. C.

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Ringle, David A., M.A., Amphibian Yolk. Student, New York University, New York, N. Y.

Roberts, Glen W., Geology, Geography and Engineering, Elizabeth, W. Va.

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Schlichting, Margaret, B.A., Veterinary Medicine. Director, Veterinary Diagnostic Laboratories, Katonah, N. Y.

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Silvestri, Luigi, M.D., Microbiology. Research Worker, Milano, Italy.

Singman, David, M.D., Experimental Nutrition. Director, Laboratories, Alta Bates Hospital, Berkeley, Calif.

Skinner, Lawrence Edwards, M.D., Medicine. Partner, Lakewood Clinic, Tacoma, Wash.

Sobelman, Sidney, M.S., Operations Research. Chief, Special Analysis Branch, Picatinny Arsenal, Dover, N. J.

Spechter, Horst-Jürgen, M.D., Medical Section. Assistant, Department of Gynecology and Obstetrics, University Tübingen, Tübingen, Germany.

Spritz, Norton, M.D., Medicine. Instructor, Medicine, Cornell University Medical College, New York, N. Y.

Staba, E. John, Ph.D., Pharmacognosy. Assistant Professor and Chairman, College of Pharmacy, University of Nebraska, Lincoln, Nebr.

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Stern, Morris Edward, M.D., Anesthesiology. Private Practice, Phoenix, Ariz.

Teir, Harald, M.D., Cancer Biology. Professor, Pathology, University of Helsinki, Helsinki, Finland.

Thomas, Wilbur Addison, M.D., Pathology. Cyrus Strong Merrill, Professor and Head, Pathology Department, Albany Medical College, Albany, N. Y.

Uribe, Pedro, Engineering. Investigation of Social Sciences, Venezuela, South America.

Van Bekkum, J. G., D.V.M., Virology. Deputy Director, Centraal Diergeneeskundig Instituut, Amsterdam, Holland.

Wood, William Holmes, Jr., A.B., Rickettsiology. Fellow in Rickettsiology, University of Maryland School of Medicine, Baltimore, Md.

Zinneman, Horace H., M.D., Immunology. Associate Professor, Department of Medicine, University of Minnesota Medical School, Minneapolis, Minn.

STUDENT MEMBERSHIP

Blaschke, Lillian A., M.A., Genetics. Graduate Student, Department of Zoology, Columbia University, New York, N. Y.

Brandon, Donald E., M.D., Internal Medicine. Resident, Bellevue Hospital, New York, N. Y.

Geller, Stephen A., B.A., Biology. Graduate Student, New York University, New York, N. Y.

Richter, Elihu, A.B., Medicine. Student, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.